(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 December 2004 (23.12.2004)

PCT

(10) International Publication Number
WO 2004/110360 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number:
PCT/US2004/015662

(22) International Filing Date: 17 May 2004 (17.05.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/471,300	16 May 2003 (16.05.2003)	US
60/471,239	16 May 2003 (16.05.2003)	US
60/471,240	16 May 2003 (16.05.2003)	US
60/474,372	29 May 2003 (29.05.2003)	US

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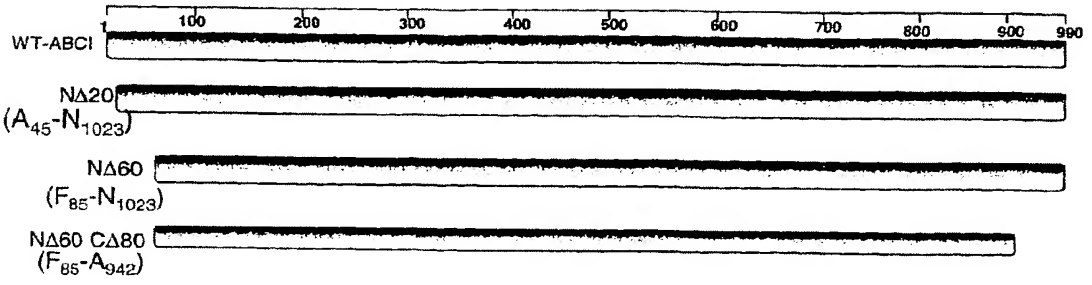
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PROTEOGLYCAN DEGRADING MUTANTS FOR TREATMENT OF CNS



(57) Abstract: The present disclosure relates to the preparation and deletion mutants of chondroitinase proteins and their use in methods for promoting the diffusion of therapeutic composition into tissues and their use for neurological functional recovery after central nervous system ("CNS") injury or disease.

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PROTEOGLYCAN DEGRADING MUTANTS FOR TREATMENT OF CNS

CROSS-REFERENCE TO RELATED APPLICATIONS

BACKGROUND AND SUMMARY

[0002] Chondroitinases are enzymes of bacterial origin that act on chondroitin sulfate, a component of the proteoglycans that are components of the extracellular matrix of a wide variety of tissues such as the central nervous system and for example they can mediate the attachment between the retina and the vitreous body of the human eye. Examples of chondroitinase enzymes are chondroitinase ABC I, **SEQ ID NO: 37**, which is produced by the bacterium Proteus vulgaris (*P. vulgaris*), and chondroitinase AC, **SEQ ID NO: 5**, which is produced by *Flavobacterium heparinum*. Chondroitinases ABC I **SEQ ID NO: 37**, and chondroitinase AC **SEQ ID NO: 5**, function by degrading polysaccharide side chains in protein-polysaccharide complexes, without degrading the protein core.

[0003] Yarnagata et al. (J. Biol. Chem. 243:1523-1535, 1968) describe the purification of the chondroitinases like ABC I **SEQ ID NO: 37** from extracts of *P. vulgaris*. This enzyme selectively degrades the glycosaminoglycans chondroitin-4-sulfate, dermatan sulfate, and chondroitin-6-sulfate (also referred to respectively as chondroitin sulfates A, B, and C which are side chains of proteoglycans) at pH 8 at higher rates than it degrades chondroitin or hyaluronic acid. The products of the degradation are high molecular weight unsaturated oligosaccharides and an unsaturated disaccharide. However, chondroitinase ABC I, **SEQ ID NO: 37**, does not act on keratosulfate, heparin or heparitin sulfate.

[0004] Uses of chondroitinases include rapid, specific and non-surgical disruption of the attachment of the vitreous body to the neural retina of the eye, thereby facilitating removal of the vitreous body.

[0005] *P. vulgaris* chondroitinase, for example ABC I **SEQ ID NO: 37** migrates with an apparent molecular mass of about 110 kDa when resolved by SDS-PAGE. The appearance of a doublet in SDS-PAGE resolution of chondroitinase ABC has been reported (Sato et al., Agric. Biol. Chem. 50:4,1057-1059, 1986). However, this doublet represents intact chondroitinase ABC and a 90 kDa degradation product. Commercial chondroitinase ABC protein preparations contain variable amounts of this 90 kDa degradation product and an additional 18 kDa degradation product also derived from chondroitinase ABC I, **SEQ ID NO: 37**.

[0006] Chondroitinase ABC II, **SEQ ID NO: 27**, has also been isolated and purified from *P. vulgaris*, Chondroitinase ABC II, **SEQ ID NO: 27**, is a polypeptide of 990 amino acids with an apparent molecular mass by SDS-PAGE of about 112 kDa. Its molecular mass as determined by electrospray and laser desorption mass spectrometry is about 111,772 daltons. Chondroitinase ABC II, **SEQ ID NO: 27**, has an isoelectric point of 8.4-8.45. Its enzymatic activity is distinct from, but complementary to, that of chondroitinase ABC I **SEQ**

ID NO: 37. Chondroitinase ABC I, **SEQ ID NO: 37**, endolytically cleaves proteoglycans to produce end-product disaccharides, as well as at least two other products which are thought to be tetrasaccharides, Chondroitinase ABC II, **SEQ ID NO: 27**, digests at least one of these tetrasaccharide products from the chondroitinase ABC I **(SEQ ID NO: 37)** digestion of proteoglycan.

[0007] After a injury in the adult mammalian central nervous system (CNS), the inability of axons to regenerate may lead to permanent paralysis. An injury-caused lesion will develop glial scarring, which contains extracellular matrix molecules including chondroitin sulfate proteoglycans (CSPGs). CSPGs inhibit nerve tissue growth *in vitro*, and nerve tissue regeneration fails at CSPGs rich regions *in vivo*.

[0008] A number of molecules, and specified regions of them, have been implicated in the ability to support the sprouting of neurites from a neuronal cell, a process also referred to as neurite outgrowth. The term neurite refers to both axon and dendrite structures. This process of spouting neurites is essential in neural development and regeneration, especially after physical injury or disease has damaged neuronal cells. Neurites elongate profusely during development both in the central and peripheral nervous systems of all animal species. This phenomenon pertains to both axons and dendrites. However, neurite regrowth in the CNS decreases as the animal's age increases.

[0009] Chondroitinase enzymes have shown efficacy in improving functional outcomes in several *in vivo* models of spinal cord injury. Recombinantly produced chondroitinases AC **(SEQ ID NO: 5)** and chondroitinase B **(SEQ ID NO: 12)** polypeptides have shown efficacy *in vitro* by overcoming the barrier of an inhibitory substrate border, such as aggrecan, resulting in neurite extension for rat cortical neurons.

[0010] The inventors have discovered through a deletion analysis based on the available crystal structures, mutant polypeptides capable of degrading chondroitin sulfate

proteoglycans (CSPGs). The cleavage activity of all these mutants have been screened *in vitro* by zymographic assay using aggrecan as a substrate. A truncated polypeptide of chondroitinase AC (nΔ50-cΔ275), **(SEQ ID NO: 11)**, lacking 50 and 275 amino acids from the amino and carboxy termini respectively and having a molecular weight of 38 kDa compared to 75kDa of the full length protein, was found to be the minimal size that retained activity as tested by a zymographic assay. The deletion mutant of chondroitinase B (nΔ120-c Δ120), **(SEQ ID NO: 17)**, lacking 120 amino acids from each of the amino and carboxy termini and having a molecular weight of 26 kDa compared to 52kDa of the full length protein, was shown to retain activity as well in a zymographic assay. Reduction in the size and complexity of the molecule may facilitate diffusion to the site of action and potentially reduce immunogenicity for prolonged therapeutic use. These smaller chondroitinases could be potential therapeutics for spinal cord injury.

[0011] The present disclosure relates to mutants of chondroitinase genes, polypeptides and proteins derived therefrom, and their use in methods for promoting neurological functional recovery after central nervous system ("CNS") injury or disease. The mutant genes, polypeptides and proteins derived from them preferably include deletion, substitution, or a combination of these from the structural units the mature gene or polypeptide; more preferably the mutant genes or polypeptides are deletion mutants of the mature gene or polypeptide. These mutant genes or polypeptides, preferably biologically active, may be used in various pharmaceutical compositions.

[0012] Polypeptide mutants of chondroitinases, for example chondroitinase ABC Type I, **SEQ ID NO: 1 or 37**, Chondroitinase ABC Type II, **SEQ ID NO: 27**, Chondroitinase AC, **SEQ ID NO: 5**, and Chondroitinase B, **SEQ ID NO: 12**, are provided. Other mammalian enzymes mutants with chondroitinase-like activity may independently include such enzymes as hyaluronidase 1, **SEQ ID NO: 30**, hyaluronidase 2, **SEQ ID NO:**

31, hyaluronidase 3, **SEQ ID NO: 32**, hyaluronidase 4, **SEQ ID NO: 33**, and optionally PH-20, **SEQ ID NO: 34**. These deletion or substitution mutant may be used alone or in combination with chondroitinases or their deletion or substitution mutants as therapeutic compositions and mixtures. Further provided is the use of these mutants, and preferably the chondroitinase deletion or substitution mutants to promote neurological functional recovery in mammals following injury to the CNS, including but not limited to contusion injury.

[0013] One embodiment of the present invention are isolated nucleic acid molecules consisting of, and preferably comprising, a nucleotide sequence encoding the amino acid sequence of polypeptides that are deletion and or substitution mutants of proteoglycan degrading molecules. Independently, nucleic acid molecules of the present invention may encode for mutant proteoglycan degrading polypeptides of chondroitinase, for example chondroitinase ABC Type I, **SEQ ID NO: 1 or 37**, Chondroitinase ABC Type II, **SEQ ID NO: 27**, Chondroitinase AC, **SEQ ID NO: 5**, and Chondroitinase B, **SEQ ID NO: 12**, hyaluronidase 1, **SEQ ID NO: 30**, hyaluronidase 2, **SEQ ID NO: 31**, hyaluronidase 3, **SEQ ID NO: 32**, hyaluronidase 4, **SEQ ID NO: 33**, or optionally PH-20, **SEQ ID NO: 34** and combinations of these. Preferably the nucleic acids encode for chondroitinase deletion and or substitution mutants, most preferably the nucleic acids encode for chondroitinase ABC type I or II polypeptides. The invention is also directed to nucleic acid molecules consisting of, and preferably comprising, a nucleotide sequence complementary to the above-described nucleic acid sequences. Also provided for are nucleic acid molecules at least 80%, preferably 85% or 90%, still more preferably 95%, 96%, 97%, 98%, or 99% identical to any of the above-described nucleic acid molecules. Also provided for are nucleic acid molecules which hybridize under stringent conditions to any of the above-described nucleic acid molecules. The present invention also provides for recombinant vectors comprising these nucleic acid molecules, and host cells transformed with such vectors.

[0014] Also provided are isolated polypeptides consisting of, and preferably comprising, the amino acid sequence of deletion and or substitution mutants of proteoglycan degrading polypeptides. Independently, proteoglycan degrading polypeptides can include chondroitinases, for example ABC Type I, **SEQ ID NO: 1 or 37**, Chondroitinase ABC Type II, **SEQ ID NO: 27**, Chondroitinase AC, **SEQ ID NO: 5**, and Chondroitinase B, **SEQ ID NO: 12**, hyaluronidase 1, **SEQ ID NO: 30**, hyaluronidase 2, **SEQ ID NO: 31**, hyaluronidase 3, **SEQ ID NO: 32**, hyaluronidase 4, **SEQ ID NO: 33**, optionally PH-20, **SEQ ID NO: 34**. Preferably the polypeptides are deletion mutants of chondroitinases. Pharmaceutical compositions may be prepared from the mutant proteoglycan degrading molecules such as these chondroitinases and or hyaluronidases; the composition may include one or more of the deletion and substitution mutants from different proteoglycan degrading polypeptides.

[0015] In one aspect of the invention, biologically active proteoglycan degrading polypeptide are provided having a deletion or substitution of at least one amino acid. The mutant proteoglycan degrading polypeptides include those having the minimal size yet retain a degree of activity as determined by the enzyme assays described in the specification. .Preferred deletion or substitution mutants of the proteoglycan degrading molecule are those which degrade chondroitin and have one or more amino acid deletions from the N-terminus, about 1 to at least about120 amino acids and/or the C-terminus, about 1 to at least about 275 amino acids, more preferably the deletions are from a chondroitinase or a substituted chondroitinase, and even more preferably chondroitinase ABC I or II or a substituted chondriotinase ABC I or II.

[0016] One aspect of this invention are deletion and or substitution mutants of proteoglycan degrading polypeptides, preferably deletion mutants of chondroitinase polypeptides, that promote neurite regeneration and or plasticity in the CNS and or promote or inhibit the diffusion of therapeutic molecules into tissues by degradation of proteoglycans.

[0017] The mutant proteoglycan degrading polypeptides, preferably deletion and or substitution mutants of chondroitinases, may promote neurite regeneration in the CNS and or promote or inhibit the diffusion of therapeutic molecules into tissues by degradation of proteoglycans and can be obtained through expression of suitably modified DNA sequences. Thus, the present invention also provides suitable expression vectors and host cells compatible with them.

[0018] In yet other aspects, the invention comprises pharmaceutical compositions that include biologically active polypeptide of deletion and or substitution mutants of proteoglycan degrading molecules, and preferably deletion or substitution mutants of chondroitn degrading polypeptides as described above, in combination with a pharmaceutically acceptable carrier.

[0019] The deletion mutants and or substitution mutants of the proteoglycan degrading polypeptides of the present invention may be used to promote the regeneration of neurites in nerve tissue. These mutants might also be useful in the treatment of other CNS disorders in which plasticity, regeneration, or both might be beneficial. For example CNS injuries and disorders may include but not limited to contusion injury, traumatic brain injury, stroke, multiple sclerosis, brachial plexus injury, ambliopia. Because of their proteoglycan degrading properties, they may be used to promote the delivery of theraprutic compositions and diagnostics to tissues and cells that are normally impermeable to them. Alternatively, they may be used to inhibit penetration of therapeutic compositions, diagnositics or cells to tissues that use part of the extracellular matrix to enter tissues. Because of their smaller size compared to the full length enzyme, the deletion and or substitution mutants are easier to make and easier to deliver to target cells and tissues. These and other even smaller deletion or substitution mutants of proteoglycan degrading molecules could be used as potential

therapeutics with lesser immunogenicity and similar or higher tissue penetration ability for the treatment of CNS injury.

[0020] The deletion mutants may offer significant advantages over the full length proteins in the therapeutic development process. The tissue penetration of the enzymes may be significantly effected by the protein size. The effect of protein size on tissue penetration is difficult to predict, but dependent on size and charge. The rate of penetration depends on tissue composition, charge interactions and hydration effects. Having active enzymes of widely ranging size may allow selection of an enzyme based on optimal tissue penetration properties, perhaps maximizing effective concentrations or limiting peripheral exposure to the enzyme.

[0021] The immune response of a mammal to a bacterial protein may or may not limit the ability to use the protein or polypeptide as a therapeutic. The generation of antibodies to the protein can restrict repeated exposures, as well as potentially inactivate the protein therapeutic making it ineffective. The smaller mutant proteoglycan degrading enzymes, preferably mutant chondroitinase enzymes, may limit the antigenic sites, limit an immune response or at least simplify the process of engineering an enzyme with reduced immunogenicity.

[0022] The release rate of proteins from matrices often used in sustained release formulations can be dependent upon size and cross-linking. The effective release rate of deletion mutants of proteoglycan degrading polypeptide from the matrix can be engineered through the manipulation of the size of the enzyme. Having a repertoire of chondroitinase enzymes of various size and charge will give an significant advantage for the development of a sustained release formulations.

A BRIEF DESCRIPTION OF THE FIGURES

[0023] FIG. 1(A) shows Anti-His-tag Western Blot (top) and zymogram (bottom) demonstrating chondroitinase B deletion NΔ120 CΔ120 mutant **(SEQ ID NO: 17)** expression activity; FIG. 1(B) shows Anti-His-tag Western Blot (top) and zymogram (bottom) demonstrating chondroitinase AC deletion NΔ50 CΔ275 mutant **(SEQ ID NO: 11)** expression activity;

[0024] FIG. 2 shows illustrates the relative substrate degrading activity of various detetion mutant polypeptides of Chondroitinase AC **(SEQ ID NO: 6-11)** relative to the full length Chondroitinase AC **SEQ ID NO: 5** ;

[0025] FIG. 3(A)shows a schematic of deletion mutant polypeptides of chondroitinase AC **(SEQ ID NO: 6-11)**; FIG. 3(B) shows confirmation of chondroitinase AC deletion mutants by Western blotting;

[0026] FIG. 4. shows confirmation of protein expression and catalytic activity of Chondroitinase AC deletion mutants **(SEQ ID NO: 6-11)**by (A) Western Blotting and (B) zymography;

[0027] FIG. 5 shows a schematic of deletion mutant polypeptides **(SEQ ID NO: 13-17)** of chondroitinase B **(SEQ ID NO: 12)**;

[0028] FIG. 6 shows confirmation of protein expression and catalytic activity of Chondroitinase B and deletion mutants **(SEQ ID NO: 12-17)** by (A) Western Blotting and (B) zymography;

[0029] FIG. 7 shows a schematic of Chondroitinase ABC I deletion mutant polypeptides **(SEQ ID NO: 2-4)** of Chondroitinase ABC I **SEQ ID NO: 1**;

DETAILED DESCRIPTION

[0030] Before the present compositions and methods are described, it is to be understood that this invention is not limited to the particular molecules, compositions, methodologies or protocols described, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0031] It must also be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to a "cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated by reference. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0032] "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event occurs or material is present and instances where the event does not occur or where the material is not present.

[0033] One aspect of the present disclosure relates to a series of deletion and or substitution mutants of chonchoitinase genes that can be used to generate deletion mutant enzymes with substantially lower molecular weight, but modified, and preferably equivalent

or superior proteoglycan degrading catalytic activity compared to the wild type enzymes. The deletion and or substitution mutants can be generated by polymerase chain reaction. The resulting mutants are expressed and then enzymatic activity of the mutant polypeptide can be confirmed by using zymography.

[0034] The mutants of the proteoglycan degrading molecules can be used to treat mammalian CNS injuries, typically caused by trauma or disease. In particular, a deletion mutant of a proteoglycan degrading polypeptide molecule like chondroitinase, for example ABC Type I, **(SEQ ID NO: 1 or 37)**, Chondroitinase ABC Type II, **(SEQ ID NO: 27)**, Chondroitinase AC, **(SEQ ID NO: 5)**, and Chondroitinase B, **(SEQ ID NO: 12)**, or mammalian enzymes with chondroitinase-like activity such as hyaluronidase 1, **(SEQ ID NO: 30)**, hyaluronidase 2, **(SEQ ID NO: 31)**, hyaluronidase 3, **(SEQ ID NO: 32)**, hyaluronidase 4, **(SEQ ID NO: 33)**, and optionally PH-20, **(SEQ ID NO: 34)**, or mixtures of any of these may be used to provide a therapeutic treatment for CNS injuries and disorders which may include but not limited to contusion injury, traumatic brain injury, stroke, multiple sclerosis, brachial plexus injury, amblioplia, spinal cord injuries. Spinal cord injuries includes disease and traumatic injuries, such as the crushing of neurons brought about by an auto accident, fall, contusion, or bullet wound, as well as other injuries. Practice of the present methods can confer clinical benefits to the treated mammal, providing clinically relevant improvements in at least one of the subject's motor coordination functions and sensory perception. Clinically relevant improvements can range from a detectable improvement to a complete restoration of an impaired or lost function of the CNS.

[0035] Mutants of proteoglycan degrading molecules, for example the deletion mutants of Chondroitinase AC **(SEQ ID NO: 5)**, may have their enzyme activity stabilized by the addition of excipients or by lyophilization. Stabilizers may include carbohydrates, amino acids, fatty acids, and surfactants and are known to those skilled in the art. Examples

include carbohydrates such as sucrose, lactose, mannitol, and dextran, proteins such as albumin and protamine, amino acids such as arginine, glycine, and threonine, surfactants such as TWEEN® and PLURONIC®, salts such as calcium chloride and sodium phosphate, and lipids such as fatty acids, phospholipids, and bile salts. The stabilizers may be added to the proteoglycan degrading polypeptide deletion mutants in a ratio of 1:10 to 4:1, carbohydrate to polypeptide, amino acids polypeptide, protein stabilizer to polypeptide, and salts to polypeptide 1:1000 to 1:20; surfactant to polypeptide; and 1:20 to 4:1, lipids to polypeptide. Other stabilizers include high concentrations of ammonium sulfate, sodium acetate or sodium sulfate, based on comparative studies with heparinase activity. The stabilizing agents, preferably the ammonium sulfate or other similar salt, are added to the enzyme in a ratio of 0.1 to 4.0 mg ammonium sulfate/IU enzyme.

[0036] The proteoglycan degrading mutant polypeptides may be formulated as compositions and can be administered topically, locally or systemically to a subject or patient. Preferably the subject is a mammal and even more preferably a human in need of a proteoglycan degrading composition such as one of the chondroitinases. Topical or local administration is can be used for greater control of application. One or more proteoglycan degrading mutant polypeptides, singularly or in combination, can be mixed with an appropriate pharmaceutical carrier prior to administration. Examples of generally used pharmaceutical carriers and additives are conventional diluents, binders, lubricants, coloring agents, disintegrating agents, buffer agents, isotonizing agents, preservants, anesthetics and the like. Specifically pharmaceutical carriers that may be used are dextran, serum albumin, gelatin, creatinine, polyethylene glycol, non-ionic surfactants (e.g. polyoxyethylene sorbitan fatty acid esters, polyoxyethylene hardened castor oil, sucrose fatty acid esters, polyoxyethylene polyoxypropylene glycot) and similar compounds.

[0037] Compositions of the present invention having a proteoglycan degrading polypeptide or a nucleic acid for expressing it may also include theraptutic molecules, diagnostics, and agents for promoting neurite growth and regeneration. Examples of diagnostic molecules may include but are not limited to fluorescent probes, radioisotopes, dyes, or magnetic contrast agents. Compounds that facilitate plasticity, neurite growth, and regeneration can include but are not limited to molecules that over come neurite out growth inhibition, or promote nerve growth such as soluble NOGO antagonists like NgR_{27-311}, neural cell adhesion molecules like L1, neurotrophic factors, growth factors, phosphodiesterase inhibitors, and inhibitors of MAG or MOG. Additionally, deletion mutants may be combined with other compounds that promote remyelination such as neuregulins (GGF2) and antibodies that promote remyelination.

[0038] Plasticity of the nervous system refers to any type of functional reorganization. This reorganization occurs with development, learning and memory and brain repair. The structural changes that occur with plasticity may include synapse formation, synapse removal, neurite sprouting and may even include strengthening or weakening existing synapses. Regeneration is generally differentiated from plasticity by the long range growth of axons in disrupted tracts that is characteristic of regeneration.

[0039] The biological activity of the proteoglycan degrading molecules of the present invention may be used to control the degradation rate of proteoglycans in a tissue, and for example be chosen to have a slower degradation activity for sensitive tissues and a higher degradation rate for degrading potions of tissue which are thicker. The activity may be contolled by one of more amino acid substitutions or deletions in the polypeptide or vectors used to express them; the activity may be controlled by the concentration or combination of proteoglycan degrading polypeptides in a composition. The proteoglycan degrading activity may be made to be greater or less than that of the full length polypeptide. For example, it can

be made to be less than that of the full length Chondroitinase AC **(SEQ ID NO: 5)**, and can be made to be less than half as active as the full length polypeptide as shown in FIG. 2. Also, as further illustrated in FIG. 2, the proteoglycan degrading activity can be made to be greater than the full length Chondroitinase AC **(SEQ ID NO: 5)**, it can be made more active than the full length polypeptide by a factor of 1.5 or more; it can be more active than the full length polypeptide by a factor of 2.5 or more.

[0040] Native or wild-type *P. vulgaris* bacterial strains typically can be used to produce chondroitinases ABC I, **(SEQ ID NO: 1 or 37)**, and chondroitinase ABC II, **(SEQ ID NO: 27)**, and mutants of these full length polypeptide under ordinary growth conditions. Wild-type strains of *P. vulgaris* can be induced to produce detectable levels of chondroitinase ABCI and its mutants by providing an inducing substrate, such as chondroitin sulfate, as the sole carbon source.

[0041] Mutant nucleic acids can be used for expressing mutant proteoglycan degrading polypeptides. The expressed polypeptides or the mutant nucleic acids can be used to treat mammalian CNS injuries, typically caused by trauma or disease. In particular, a deletion mutant nucleic acid for expressing proteoglycan degrading polypeptide molecule like chondroitinase ABC Type I, may include but are not limited to cloned chondroitinase ABC I, **(SEQ ID NO: 22 or 28)**, chondroitinase ABC II, **(SEQ ID NO: 26)**, nucleic acids for expressing fusion proteins of deletion mutants TAT-chondroitinase ABC I NΔ60 **(SEQ ID NO: 43)** and mutants of these genes in *E. coili* can be expressed using a heterologous expression system with an artificial inducer. Chondroitinase AC **(SEQ ID NO: 22 or 28)**, and chondroitinase B **(SEQ ID NO: 26)**, and their mutants may be cloned from *F. heparinum* and expressed in *E. coli*.

[0042] The full length proteoglycan degrading molecules like Chondroitinase AC **(SEQ ID NO: 5)**, as well as the deletion and or substitution mutants of the proteoglycan

degrading polypeptides may be cloned in a number of bacterial as well as mammalian expression vectors. Non-limiting of these vectors include pET15b, pET14b, pGEX 6P1, pDNA4HisMax, or pSECTag2b. The deletion mutants and substituted polypeptides of the present invention exhibit the ability to degrade proteoglycans such as chondroitin CS and DS, and have a smaller size and molecular weight than the mature enzyme polypeptides which is expected to facilitate their diffusion into cells, tissues and across membranes. Expression vectors can include the nucleic acid sequence that expresses a mutant proteoglycan degrading polypeptide operably linked to an expression control sequence. Operably linked can refer to a linkage between an expression control sequence and coding sequence, where the linkage permits the expression control sequence to control the expression of the coding sequence.

[0043] The properties of the naturally occurring, substituted and or deletion mutants of the proteoglycan degrading molecules may be altered by introducing a variety of mutations in the protein. Such alterations are suitably introduced using the mutagenesis techniques, for example but not limited to PRC mutagenesis, and the mutated polypeptides molecules suitably synthesized using the expression vectors.

[0044] Mutant proteoglycan degrading polypeptides of the present invention include deletions and or substitutions of amino acids from mature proteoglycan degrading polypeptides. Preferably the deletions or substitutions include any two consecutive or separated amino acids, N or C terminal amino acid deletions or substitutions, and internal amino acid deletions or substitutions in the polypeptide. The deletions and or substitutions can start with any amino acid in the molecule and it is possible to have two separated deletions in the molecule. The deletion or substitution results in mutant proteoglycan degrading polypeptide that are smaller than the mature enzyme and retain proteoglycan degrading ability. Mutant proteoglycan degrading polypeptides can be fused or linked to another polypeptide. Polypeptide is used to unambigously encompases amino acid sequences

for mutants of any length which have proteoglycan degrading activity and improve plasticity including those minus the signal sequence that is initially part of polypeptide when it is translated and that is cleaved off by a host-translational modification.

[0045] Mutant nucleic acids of the present invention include deletions and or substitutions of nucleotides from genes which express the mature proteoglycan degrading polypeptides. The deletion and substitution mutations at the DNA level are used to introduce amino acid substitutions and or deletions into the encoded protein. These nucleotide deletions and substitutions can be used to introduce deletions and or substitutions into important conformational or active regions of the polypeptide. A nucleic acid fragment is a nucleic acid having fewer nucleotides than the nucleotide sequence encoding the entire amino acid sequence of a mature proteoglycan degrading polypeptide, yet which preferably encodes a mutant polypeptide which retains some biological activity of the full length protein, e.g., the expressed polypeptide fragment retains the ability to induce degradation of proteoglycans, promote diffusion of therapeutics into cells and tissue, or promote regeneration of neurites. Genes encoding either N or C terminal mutants of proteoglycan degrading polypeptide domains linked to other polypeptides can also be used in constructs for expression of fusion proteins linked to mutant proteoglycan degrading polypeptides.

[0046] The deletion and or substitution mutant proteoglycan degrading polypeptides of the present invention may also include derivatives of these polypeptides which have been been chemically or enzymatically modified, but which retain their biological activity to degrade proteoglycans. The proteoglycan degrading activity of these mutants may be controlled depending upon the deletion and or substitution made to the polypeptide or the nucleic acid used to express the polypeptide. Variants, fragments, or analogs of the mature proteoglycan degrading polypeptides or nucleic acids and vectors used to express them include mutant polypeptides and nucleic acids having a sequence which differs from the

mature polypeptide or nucleic acid sequence by one or more deletions, substitutions, or a combination of both such that the mutant proteoglycan degrading polypeptides retain their biological activity and can degrade proteoglycans, and preferably degrade chondroitin sulfate proteoglycans.

[0047] Due to the degeneracy of the genetic code, one of ordinary skill in the art will recognize that a large number of the nucleic acid molecules having a sequence at at least 80%, preferably 85% or 90%, still more preferably 95%, 96%, 97%, 98%, or 99% identical to a nucleic acid sequence encoding for a mutant proteoglycan degrading molecule will encode a mutant polypeptide having proteoglycan degrading activity and preferably chondroitin degrading ability. It will be further recognized that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a mutant polypeptide having proteoglycan degrading activity. This is because amino acid substitutions that are either less likely or not likely to significantly effect polypeptide activity (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid) to degrade proteoglycans and preferably to degrade chondroitin.

[0048] Variants included in the invention may contain individual substitutions, deletions or additions to the nucleic acid or polypeptide sequences. Such changes will alter, add or delete a single amino acid or a small percentage of amino acids in the encoded sequence. Variants are referred to as "conservatively modified variants" where the alteration results in the substitution of an amino acid with a chemically similar amino acid.

[0049] The discovery that the proteoglycan degrading activity of the deletion and substitution mutant polypeptides of the present invention can be controlled to be less, about the same, or greater than the full length proteoglycan degrading molecule has another potential advantage. A pharmaceutical composition containing the proteoglycan degrading molecules may be administered parenterally, intravenously or subcutaneously. The use of a

hydrogel composed of biodegradable polymer enclosing the polypeptide and continuously releasing the polypeptide is limited by the amount of polypeptide that can be enclosed in the hydrogel. Using a deletion mutant of the polypeptide with higher specific activity implies that, on a molar basis, more of the active substance can be enclosed in the same volume, thereby increasing the time between successive administrations or possibly avoiding repeated administrations.

[0050] Purification of the polypeptide obtained after expression is dependent on the host cell and the expression construct used. Generally, the purification of proteoglycan deletion or substitution mutants can be performed in the same way as the purification of native full length polypeptides including the use of histidine-tags.

[0051] The deletion or substitution mutant proteoglycan degrading polypeptides and proteins are administered in an amount effective to degrade CSPGs. The polypeptides may be used to aid the diffusion of therapeutic and diagnostic compositions to tissues and and can be used to promote the recovery of neurological function and neurite outgrowth. Once the mutant proteoglycan degrading proteins or polypeptides in the compositions have been purified to the extent desired, they may be suspended or diluted in an appropriate physiological carrier or excipient for SCI treatment or for screening assays of compositions promoting neurite growth *in vitro* on suitable substrates like aggrecan. In models of SCI, effective intrathecal doses of chondroitinases in rats have been about 0.06 units on alternate days for 14 days. A dose for a 70 kilogram human may be about 17 Units. At about 100 Units / milligram, this would equal about 170 micrograms. Doses of up to 20 Units appear safe in mammalian subjects like rats. Compositions may include a proteoglycan degrading mutant polypeptide, preferably mutant chondroitinase polypeptides, and more preferably still deletion mutant chondroitinase polypeptides. These compositions may also include other proteoglycan degrading molecules and deletion and or substitution mutants of them,

molecules which block the action of neurite growth inhibitors, molecules which promote neurite or axon adhesion, diagnostic, therapeutic, or the proteoglycan degrading molecule mutant as part of a fusion protein. The mixture or fusion protein may be added to a carrier or pharmaceutically acceptable excipient can be injected, generally at concentrations in the range of 1 ug to 500 mg/kg of subject. Administering the agent can be by bolus injection, intravenous delivery, continuous infusion, sustained release from implants, or sustained release pharmaceuticals. Administration by injection, can be intramuscularly, peritoneally, subcutaneously, intravenously, intrathecally. Oral administration may include tablets or capsules, preferably the oral dosage is a sustained release formulation for once or twice daily administration. Percutneous administration can be once per day, and is preferably less than once per day administration. Administration to the human patient or other mammalian subject may be continued until a measurable improvement in autonomic or motor function in the patient is achieved.

[0052] The mutant proteoglycan degrading polypeptides or fusion polypeptides that include them may also be expressed or secreted by genetically modified cells. The expressed deletion or substitution proteoglycan degrading polypeptide or fusion polypeptides may be harvested and purified for a therapeutic compositon, or the genetically modified cells can be implanted, either free or in a capsule, at or near the site of CNS injury or a tissue into which the controlled diffusion of therapeutic or diagnostic molecule is desired. Mutant nucleic acids for expressing mutant proteoglycan degrading polypeptides are illustrated by non-limiting examples of chondroitinase ABC I **(SEQ ID NO: 22 and 28)** which encode for substituted chondroitinase ABC I polypeptides and those without leader amino acid sequences; chondroitinase B nucleic acid mutant **(SEQ ID NO: 21)** which encodes for mutant polypeptide NΔ120 CΔ120 of chondroitinase B **(SEQ ID NO: 21)**; and chondroitinase AC nucleic acid mutant **(SEQ ID NO: 19)** which encodes for mutant

polypeptide NΔ50 CΔ275 of chondroitinase AC **(SEQ ID NO: 11)**. A non-limiting example of a fusion nucleic acid includes a TAT-deletion mutant chondroitinase ABCI fusion DNA construct **(SEQ ID NO: 41)**. Another example would be a nucleic acid for TAT-chondroitinase ABCI-NΔ60 **(SEQ ID NO: 43)** for the expressed polypeptide **(SEQ ID NO: 44)**.

[0053] Once the mutant proteoglycan degrading polypeptide are administered to cells or a tissue with CSPGs, degradation of CSPGs removes the inhibitory molecules that block neurite outgrowth, and allow the regeneration of neurites into the affected area. The removal of CSPG also promotes plasticity in the CNS. For example, the full length polypeptides of chondroitinase AC **(SEQ ID NO: 5)**, and chondroitinase B, **(SEQ ID NO: 12)**, degrade CS and DS, respectively, resulting in unsaturated sulfated disaccharides. Chondroitinase AC **(SEQ ID NO: 5)**, cleaves CS at 1, 4 glycosidic linkages between N-acetylgalactosamine and glucuronic acid in the polysaccharide backbone of CS. Cleavage occurs through beta-elimination in a random endolytic action pattern. Chondroitinase B **(SEQ ID NO: 12)** cleaves the 1, 4 galactosamine iduronic acid linkage in the polysaccharide backbone of DS. The cleavage of both CS and DS occurs through a beta-elimination process which differentiates these enzymatic mechanisms from mammalian GAG degrading enzymes. Chondroitinase ABC I **(SEQ ID NO: 1)**, chondroitinase ABC II **(SEQ ID NO: 27)**, are exo and endo lyases that cleave both CS and DS. The removal of CS and DS from a glial scar permits the regeneration of neurite outgrowths into the injured area and promotes plasticity. For example, the proteoglycan degrading molecules illustrated in FIG. 2, Chondroitinase AC **(SEQ ID NO: 5)** and various mutant Chondroitinase AC **(SEQ ID NO: 6-11)** degrade a model proteoglycan substrate at by various amounts. Similar results are shown by *in vitro* zymograph for chondroitinase B **(SEQ ID NO: 12)** and illustrative mutants **(SEQ ID NO: 13-17)** in FIG. 6. It is reasonable to expect that since a proteoglycan

degrading molecule like Chondroitinase ABC I **(SEQ ID NO: 1)** improves functional recovery in rats with contusive spinal cord injury and also facilitates the diffusion of model compounds into brain tissue, that mutant proteoglycan degrading polypeptides and compositions containing them can also improve functional recovery in mammalian subjects like rats with contusive spinal cord injury and may also facilitates the diffusion of model compounds into brain tissue.

[0054] The regeneration of the nerve cells and restoration of plasticity in the affected CNS area allows the return of motor and sensory function. Clinically relevant improvement will range from a detectable improvement to a complete restoration of an impaired or lost nervous function, varying with the individual patients and injuries. The degree of functional recovery can be demonstrated by improved corticospinal tract conduction, improved tape removal, beam walking, grid walking and paw placement following chondroitinase treatment of a dorsal column lesion. Motor skill improvement as well as autonomic function: bowel, bladder, sensory and sexual function may also be used as measures of function improvement and related to molecular structure and components in the compositions of the present invention.

[0055] A series of polynucleotides that include coding for deletion or substition mutants of proteoglycan degrading polypeptides may be generated by PCR using the full length cDNAs for the proteoglycans as templates and cloned into an expression vector such as pET15b at the NdeI and BamHI sites for expression in *E. Coli.* After induction of gene expression with isopropyl-β-D-thiogalactopyranoside (IPTG), the bacteria can lysed by sonication with the concomitant extraction of the mutant polypeptide with a surfactant such as Triton X-114/PBS. The majority of recombinant proteoglycan degrading polypeptide may be found in the cytosolic fraction of the bacterial cell lysate and chondroitinase purification protocols can be used to obtain the mutant proteoglycan degrading enzyme with high activity

at high yields. This protocol may include purification by a column having anti-His antibody to selectively bind His-tagged mutant proteoglycan degrading polypeptides and may also includes cation-exchange chromatography as a capture step and gel filtration as a polishing step. After these steps, anion exchange membrane filtration, for example Intercept Q, Millipore, can be used for endotoxin and host DNA removal. Following filtration, the proteoglycan degrading mutant polypeptides can be dialyzed into volatile buffer, pH 8.0 and lyophilized to dryness. The final product is expected to be stable at -70°C for long term storage. The pI of the purified basic proteoglycan degrading mutant polypeptide may be determined by IEF-PAGE analysis of the samples from the crude cell lysate.

[0056] A variety of analytical methods can be used to compare the enzymatic activity of the recombinant version the deletion or substitution mutants of proteoglycan degrading polypeptides with those of full length proteoglycan degrading molecules like chondroitinase ABC I **(SEQ ID NO: 37)** or a commercially available form of the enzyme. The methods may also be adapted to evaluate the activity of fusion proteins including a mutant proteoglycan degrading polypeptide portion. Specific activity measurements may be obtained using an accepted spectrophotometric assay that measures the change in absorbance due to the production of reaction products from the degradation of proteoglycans. Size exclusion chromatography can be used to compare the hydrodynamic properties of the mutant enzymes.

[0057] A form of zymography can used to characterize the mature proteoglycan degrading enzyme and may be adapted for characterization of the mutants proteoglycan degrading polypeptides. Polyacrylamide gels can be polymerized in the presence of aggrecan, a substrate for proteoglycan degrading molecules like chondroitinase ABCI. The mutant proteoglycan degrading polypeptides, enzyme samples, may be resolved on the aggrecan-impregnated gels by electrophoresis in the presence of SDS. The gels can then be

subjected to a renaturation step wherein the SDS can be extracted and the enzymes allowed to refold. The refolded enzyme regains activity then digests aggrecan within the gel and the resulting loss of carbohydrate in that region of the gel that can be visualized by a carbohydrate-specific stain. A similar loss of carbohydrate in the gel would be expected for equally active forms and concentration of the mutant proteoglycan degrading molecules. In the case of recombinant Chondroitinase ABCI, its activity can be visualized as a clear spot in the zymogram. The zymography results are consistent with the spectrophotometric analysis.

[0058] HPLC methods may be used for detecting the four and six sulphated disaccharides (Δ4DS and Δ6DS, respectively) liberated as a result of mutant proteoglycan degrading polypeptide digestion of CSPG. The two disaccharides can be effectively resolved by anion exchange chromatography. The HPLC assay for the quantitation of Δ4DS and Δ6DS from chromatograms is expected to yield a linear relationship proportional to the amounts injected into the HPLC. Production of Δ4DS and Δ6DS from CSPG digestion is directly related to the amount of chondroitinase specific activity as determined by the spectrophotometric assay. This assay may be used as a sensitive and accurate method to independently quantitate Δ4DS and Δ6DS released by mutant proteoglycan degrading polypeptide digestion of a variety of substrates and may also be used to determine the activity of mutant proteoglycan degrading polypeptides and fusion proteins including them.

[0059] Another functional assay that can be performed to characterize mutant proteoglycan polypeptide activity is where dorsal root ganglian (DRG) neurons are plated on aggrecan or aggrecan treated with a deletion or substitution mutant proteoglycan degrading polypeptide . It is expected that neurons plated on aggrecan will fail to adhere to the plate and extend axons. In contrast, neurons plated on aggrecan treated with a mutant proteoglycan degrading polypeptide in a composition or as part of a fusion polypeptide would be expected to adhere to the surface and extend axons. The extensive axon growth, which is observed for

chondroitinase ABC I **(SEQ ID NO:37)** is believed to be due to the digestion of the carbohydrates on the aggrecan core protein which creates a more permissive substrate for axon growth.

[0060] Various aspects of the invention may be understood with reference to the following non-limiting examples.

EXAMPLE 1

[0061] This phrophetic example illustrates the diffusion of molecules into cells and tissue using a deletion or substitution mutant of a proteoglycan degrading polypeptide in a composition.

[0062] A brain from an adult Sprague Dawley rat may be removed from the skull and hemispheres may be soaked in buffer alone or containing about 33U/ml of a mutant proteoglycan degrading polypeptide such as **(SEQ ID NO: 9) NΔ50 CΔ200 AC (T_{74}-T_{500}) protein** for 2 hours at 37 °C. Hemispheres can be rinsed and immediately placed in dye such as Eosin Y (Sigma) or a saturated solution of Congo Red (Sigma) in 70% ethanol. Slabs of tissue may be cut and images acquired on a scanner. The penetration of the dyes into the brain tissue may be used as an indication of the proteoglycan degrading activity of a mutant proteoglycan degrading molecule and expectant penetration or diffusion of therapeutic and diagnostic molecules into the same type of tissue.

EXAMPLE 2

[0063] This prophetic example illustrates a Chondroitinase ABC I Assay Protocol which may be modified to measure the activity of a mutant proteoglycan degrading molecule,

for example a Chondroitinase ABCI deletion mutant or a fusion proteins including a deletion and or substitution mutant of a proteoglycan degrading polypeptide.

[0064] The production of reaction products from the catalytic activity of a proteoglycan degrading molecule or fusion protein can be determined by a measurement of the absorbance of the proteoglycan degradation product at a wavelength of 232 nm. A typical reaction mixture consisted of 120 µl of reaction mixture (40mM Tris, pH 8.0, 40mM NaAcetate, 0.002% casein) combined with a substrate (5 µl of 50 mM chondroitin C (MW 521), chondroitin 6 SO_4, or dermatan sulfate) and 1.5 µl of chondroitinase ABCI **(SEQ ID NO:1)** or a mutant of chondroitinase like **(SEQ ID NO:2)**. Reaction mixture aliquots of about 120 µl can be prepared at 30-37°C for 3 min or longer. The product formation is monitored as an increase in absorbance at 232 nm as a function of time at a wavelength of 232 nm using a spectrometer. The reaction may be stopped by addition of 0.1% SDS followed by boiling for 5 minutes. The observed activity may be converted to units (µmoles of product formed per minute) using the molar absorption coefficient for the C4-C5 double bond formed in the reaction (3800 $cm^{-1}min^{-1}$).

[0065] Knowing the molar absorption coefficient for the reaction product, measuring the change in the absorbance of the reaction product at 232 nm reading over time upon addition of a known amount of the Chondroitinase ABCI **(SEQ ID NO:1)** or other other mutant proteoglycan degrading polypeptide to the 120 µl reaction mixture with 0002% casein and a chondroitin substrate added, the specific activity in umol/min/mg of the mutant proteoglycan degrading polypeptide can be determined. Seikagaku Chondroitinase ABC I has a specific activity under these assay conditions of about 450 µmole/min/mg.

[0066] A proteoglycan degrading molecule like Chondroitinase ABC I **(SEQ ID NO:37)**, digests axon growth inhibiting chondroitin present in CNS tissue and improves functional recovery in rats having contusion spinal cord injuries. It is reasonable to expect

that mutants of proteoglycan degrading molecules, such as **(SEQ ID NO: 11) NΔ50 CΔ275 AC (T_{74}-T_{426}) polypeptide** that show proteoglycan degrading activity may also show some regeneration of nerves, stimulate plasticity and be useful for diffusion of agents into tissues. The mode of administration, the timing of administration and the dosage are carried out such that the functional recovery from impairment of the CNS is enhanced by the promotion of neurite outgrowth and plasticity. It is reasonable to expect that once the deletion or substitution mutants of proteoglycan degrading molecules such as **(SEQ ID NO: 11) NΔ50 CΔ275 AC (T_{74}-T_{426}) protein** are administered, the degradation of CSPGs can remove the inhibitory molecules in tissue that block drug diffusion, block neurite outgrowth, and promote the regeneration of neurites or other therapeutics into the affected area. The regeneration and plasticity of the nerve cells into the affected CNS area may allow the return of motor and sensory function. Clinically relevant improvements will range from a detectable improvement to a complete restoration of an impaired or lost nervous function, varying with the individual patients and injuries.

EXAMPLE 3

[0067] This example shows that deletion mutants of chondroitinase are biologically active.

[0068] Recombinantly produced chondroitinases AC and B have shown efficacy *in vitro* by overcoming the barrier of an inhibitory substrate border, such as aggrecan and result in neurite extension for rat cortical neurons. To facilitate effective transport of the above enzymes to the injury site, deletion mutants of these chondroitinases were prepared to determine the minimally-sized polypeptides capable of degrading CSPGs. The cleavage activity of all these mutants have been screened *in vitro* by zymographic assay using aggrecan as substrate. A truncated polypeptide of chondroitinase AC (nΔ50-cΔ275) **(SEQ**

ID NO:11) lacking 50 and 275 amino acids from the amino and carboxy terinini respectively having a molecular weight of 38 kDa compared to 75kDa of the full length protein was found to be about the minimal size mutant chondroitinase AC that retains activity as tested by zymography assay FIG. 4(B). However, an even smaller mutant, the deletion mutant of chondroitinase B (nΔ 120-cΔ 120) **(SEQ ID NO:17)** lacking 120 amino acids from each of the amino and carboxy termini, having a molecular weight of 26 kDa compared to 52 kDa of the full length protein has also shown to retain activity as well in zymography assay FIG. 6(B). These and other even smaller deletion mutants could be used as potential therapeutics with lesser immunogenicity and similar or higher tissue penetration ability compared to the mature enzyme and may be used for treatment of spinal cord injury.

[0069] A series of chondroitinase AC and B deletion mutants were generated by PCR using the full-length cDNAs for chondroitinases AC and B as templates and cloned in the pET15b expression vector at the NdeI and BamHI sites. Full length and deletion mutants were constructed with Histidine-tags for ease of detection and purification. Each of these cDNAs was induced by Isopropyl-β-D-Thiogalactopyranoside (IPTG,) and the expression was confirmed by Western blotting using anti-His antibody (Novagen). FIG. 3(A) show various non-limiting deletion mutants schematically, and FIG 3(B) shows confirmation of expression of these chondroitinase AC mutant polypeptides by anti-histidine tag Western blotting. Figures 5 and 6 show the same information for chondroitinase B deletions. Western blots demonstrate proteins of predicted size. Zymographic PAGE of deletion mutants show intense bands of substrate digestion (light) and negative carbohydrate staining.

[0070] Zymography assay. SDS-polyacrylamide gels were poured with aggrecan (85 μg/ml) polymerized into it. Crude extracts of deletion mutants of chondroitinases AC and B were run and renatured at 37 °C overnight. After separation the gel is incubated in 0.2% Cetylpyridinium for 90 minutes at room temperature. The digestion of the

proteoglycans by the chondroitinases is visualized by staining the gel with 0.2% Toludene Blue in ethanol-H_2O-acetic acid (50:49:1 v/v/v) for 30 minutes and destained with ethanol-H_2O-acetlc acid (50:49:1 v/v/v). Following destaining the gel is incubated overnight in a 50 μg/ml solution of Stains-all in 50% ethanol in the dark and destained with H_2O. Appearance of clear bands on the gel shows the digestion of carboyhydrates by the chondroitinases of the CSPG leaving the core protein which remains unstained (FIG 4.and FIG. 6).

EXAMPLE 4

[0071] This example describes the linking of a His tag to a mutant proteoglycan degrading polypeptide.

[0072] Deletion mutants of the chondroitinase ABC I enzyme where the mutant is missing a certain number of amino acids from the N-terminal and maintains proteoglycan degrading activity can be generated **(SEQ ID NO:2-4)**. These N-terminal deletion maintain a histidine-tag that is attached to the N-terminus; however similarly tagged full length chondroitinase ABC I **(SEQ ID NO:1)** did not maintain the histidine-tag after experession.

[0073] Catalytically active deletion mutants of chondroitinase ABC I **can be** prepared for example but not limited to deleting 20, and 60 amino acids respectively from the N-terminus of the mature ABC I protein as shown in FIG. 7. A mutant polypeptide with both N and C terminal deletions such as chondroitinase ABC I-NΔ60-CΔ80 **(SEQ ID NO:4)** can also be made.

[0074] These chondroitinase deletion mutants and mutants of other proteoglycan degrading molecules may used for construction of N-terminal fusion chimeric protein. Assay tests with these fusion polypeptides for chondroitin degradation and may be used to determine the efficacy of mature ABCI versus various deletion mutant in compositions and fusion proteins with respect to the substrate specificity, substrate binding and tissue

penetration. Functional assay that can be performed to characterize the activity of mutant proteoglycan polypeptide or fusion polypeptides including them. In this functional assay, dorsal root ganglian (DRG) neurons can be plated on aggrecan or aggrecan treated with a mutant proteoglycan degrading polypeptide or a fusion polypeptide including the mutant . It is expected that neurons plated on aggrecan will failed to adhere to the plate and extend axons. In contrast, neurons plated on aggrecan treated with a mutant proteoglycan degrading polypeptide or a fusion polypeptide including the mutant in a composition or as part of a fusion polypeptide would be expected to adhere to the surface and extend axons. The extensive axon growth, which is observed for a chondroitinase like chondroitinase ABC I **(SEQ ID NO: 1 or 37)** treated aggrecan substrate is believed to be due to the digestion of the carbohydrates on the aggrecan core protein which creates a more permissive substrate for axon growth.

EXAMPLE 5

[0075] This phrophetic example describes a mutant of chondroitinase ABC I that has native protein structure, but lacks proteoglycan degrading catalytic activity.

[0076] This mutant may be prepared as a null or a negative control for bioassays and SCI studies. Based on the crystal structure of chondroitinase ABC I a site-specific mutant designated H501a and Y508a **(SEQ ID NO: 36)** to knock out catalytic activity in the putative active site can be prepared. Such mutants can be tested for inactivation of catalytic activity and SEC to compare to the wild-type enzyme. The null activity mutant can also be used to provide a negative control for the various proteoglycan degrading fusion proteins for use in bioassays and ultimately in SCI animal studies.

EXAMPLE 6

[0077] This example illustrates examples of mutant proteoglycan degrading polypeptides that include both substitution and deletions from polypeptides of the present invention.

[0078] The chondroitinase ABC I sequence **(SEQ ID NO: 37)** is a published sequence for a mature chondroitinase ABC I peptide and includes the leader sequence. Chondroitinase ABC I sequence **(SEQ ID NO: 37)** is similar to **(SEQ ID NO: 1 or 29)**, however **(SEQ ID NO: 1)** does not have the first 25 amino acids of **(SEQ ID NO: 37)**, and amino acids at positions 154 and 195 of **(SEQ ID NO: 37)** differ from those (substitutions) found in similar positions when **(SEQ ID NO: 1)** and **(SEQ ID NO: 37)** are aligned.

[0079] **(SEQ ID NO: 38-40)** illustrate deletions from either the N or C terminal of the **(SEQ ID NO: 37)** polypeptide and substitutions relative to **(SEQ ID NO: 1)**. These mutant polypeptides are NΔ20 **(SEQ ID NO: 38)**, NΔ60 **(SEQ ID NO: 39)** and NΔ60 CΔ80 **(SEQ ID NO: 40).**

EXAMPLE 7

[0080] This example illustrates non-limiting illustrations of mutant polypeptides of the present invention fused with a membrane transduction polypeptide such as but not limited to a polypeptide portion of a HIV TAT protein. Full sequence listings for the mutants fusion polypeptides are provided in the Sequence listing included in the specification.

[0081] A nucleotide sequence for TAT-chondroitinase ABCI-nΔ20 **(SEQ ID NO. 41)**, a portion of which is illustrated below, shows the TAT sequence nucleotides highlighted by underlining linked to chondroitinase nucleotides.

1 ggtc gtaaaaagcg tcgtcaacgt cgtcgtcctc ctcaatgcgc acaaaataac

61 ccattagcag acttctcatc agataaaaac tcaatactaa cgttatctga taaacgtagc

[0082] The underlined nucleotides in this portion of the nucleic acid sequence denote a TAT sequence attached to the 5' of chondroitinase ABC I-NΔ20 nucleic acid **(SEQ ID NO. 47)**.

[0083] An amino acid sequence for TAT-chondroitinase ABCI-nΔ20 **(SEQ ID NO. 42)**, a portion of which is shown below, illustrates the TAT sequence amino acids highlighted by underlining at the N-terminus of chondroitinase ABCI-NΔ20 **(SEQ ID NO. 2)**.

grkkrrqrrrppqcaqnnpladfssdknsiltlsdkrsimgnqsllwkwkggssftlhkklivptdkeaskawgrsstpvfsfwly
nekpidgyltidfgeklistseaqagfkvkldftgwrtvgvslnndlenremtlnatntssdgtqdsigrslgakvdsirfkapsnvsq
geiy

[0084] A nucleotide sequence for TAT-ABCI-NΔ60 **(SEQ ID NO. 43)**, a portion of which is illustrated below, shows the N-terminal TAT **(SEQ ID NO. 49)** nucleotides highlighted by underlining.

ggtcgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgctttactttacataaaaaactgattgtccccaccgataaagaagcatcta
aagcatggggacgctcatccacccccgttttctcattttggctttacaatgaaaaaccgattgatggttatcttactatcgatttcgg.....

[0085] Amino acid sequence for TAT-ABCI-nΔ60 **(SEQ ID NO. 44)** a portion of which is shown below, illustrates the TAT sequence **(SEQ ID NO. 50)** highlighted by underlining at the N-terminus of chondroitinase ABC I -NΔ60 **(SEQ ID NO. 3)**.

grkkrrqrrrppqcftlhkklivptdkeaskawgrsstpvfsfwlynekpidgyltidfgeklistseaqagfkvkldftgwrtvgvsl
nndlenremtlnatntssdgtqdsigrslgakvdsirfkapsnvsqgeiyidrimfsvddaryqwsdyqvktrlsepeiqf....

[0086] Nucleotide sequence for ABCI-TAT-C **(SEQ ID NO. 45)**, a portion of which is illustrated below, shows the C-terminal TAT sequence nucleotides highlighted by underlining. The stop codon from chondroitinase ABC I **(SEQ ID NO. 28)** was replaced by the TAT sequence and was placed at the 3'end of the TAT sequence.

...gattaatggcaaatggcaatctgctgataaaaatagtgaagtgaaatatcaggtttctggtgataacactgaactgacgtttacgagtt
actttggtattccacaagaaatcaaactctcgccactccct ggtcgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgctag

[0087] Amino acid sequence for ABCI-TAT-C **(SEQ ID NO. 46)**, a portion of which is shown below, illustrates the TAT sequence, highlighted by underlining, linked to the chondroitinase polypeptide at the C-terminus of the mature chondroitinase ABC I **(SEQ ID NO. 1)**.

...aekvnvsrqhqvsaenknrqptegnfssawidhstrpkdasyeymvfldatpekmgemaqkfrennglyqvlrkdkdvhi
ildklsnvtgyafyqpasiedkwikkvnkpaivmthrqkdtlivsavtpdlnmtrqkaatpvtinvtingkwqsadknsevkyq
vsgdnteltftsyfgipqeiklsplpgrkkrrqrrrppqc

EXAMPLE 8

[0088] This example illustrates the sequence of chondroitinase nucleic acid and polypeptides which may be used for deletions or substitutions in mutants of the present invention. In these sequence, discrepancies from published sequences are highlighted in bold text at both the nucleotide level and at the amino acid level. These are illustrative of substitutions in the present invention.

SEQ ID NO: 26 Present invention Chondroitinase ABC II Nucleic acid

```
>_ ABC II mature                                          2973 nt vs.
>_ ABC II (present invention)                             2974 nt
scoring matrix: , gap penalties: -12/-2
99.0% identity;          Global alignment score: 11684

               10         20         30         40         50         60
806559 TTACCCACTCTGTCTCATGAAGCTTTCGGCGATATTTATCTTTTTGAAGGTGAATTACCC
       :::::::::::::::::::::::::::::::::::::::::::::::::::: :::::::::
_      TTACCCACTCTGTCTCATGAAGCTTTCGGCGATATTTATCTTTTTGAAGGCGAATTACCC
               10         20         30         40         50         60

               70         80         90        100        110        120
806559 AATACCCTTACCACTTCAAATAATAATCAATTATCGCTAAGCAAACAGCATGCTAAAGAT
       :::: ::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      AATATCCTTACCACTTCAAATAATAATCAATTATCGCTAAGCAAACAGCATGCTAAAGAT
               70         80         90        100        110        120
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```
                130        140        150        160        170        180
806559 GGTGAACAATCACTCAAATGGCAATATCAACCACAAGCAACATTAACACTAAATAATATT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GGTGAACAATCACTCAAATGGCAATATCAACCACAAGCAACATTAACACTAAATAATATT
-              130        140        150        160        170        180

                190        200        210        220        230        240
806559 GTTAATTACCAAGATGATAAAAATACAGCCACACCACTCACTTTTATGATGTGGATTTAT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GTTAATTACCAAGATGATAAAAATACAGCCACACCACTCACTTTTATGATGTGGATTTAT
-              190        200        210        220        230        240

                250        260        270        280        290        300
806559 AATGAAAAACCTCAATCTTCCCCATTAACGTTAGCATTTAAACAAAATAATAAAATTGCA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       AATGAAAAACCTCAATCTTCCCCATTAACGTTAGCATTTAAACAAAATAATAAAATTGCA
-              250        260        270        280        290        300

                310        320        330        340        350        360
806559 CTAAGTTTTAATGCTGAACTTAATTTTACGGGGTGGCGAGGTATTGCTGTTCCTTTTCGT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       CTAAGTTTTAATGCTGAACTTAATTTTACGGGGTGGCGAGGTATTGCTGTTCCTTTTCGT
-              310        320        330        340        350        360

                370        380        390        400        410        420
806559 GATATGCAAGGCTCTGTGACAGGTCAACTTGATCAATTAGTGATCACCGCTCCAAACCAA
       ::::::::::::::::: ::::::::::::::::::::::::::::::::::::::::::::::
       GATATGCAAGGCTCTGCGACAGGTCAACTTGATCAATTAGTGATCACCGCTCCAAACCAA
-              370        380        390        400        410        420

                430        440        450        460        470        480
806559 GCCGGAACACTCTTTTTTGATCAAATCATCATGAGTGTACCGTTAGACAATCGTTGGGCA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GCCGGAACACTCTTTTTTGATCAAATCATCATGAGTGTACCGTTAGACAATCGTTGGGCA
-              430        440        450        460        470        480

                490        500        510        520        530        540
806559 GTACCTGACTATCAAACACCTTACGTAAATAACGCAGTAAACACGATGGTTAGTAAAAAC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GTACCTGACTATCAAACACCTTACGTAAATAACGCAGTAAACACGATGGTTAGTAAAAAC
-              490        500        510        520        530        540

                550        560        570        580        590        600
806559 TGGAGTGCATTATTGATGTACGATCAGATGTTTCAAGCCCATTACCCTACTTTAAACTTC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TGGAGTGCATTATTGATGTACGATCAGATGTTTCAAGCCCATTACCCTACTTTAAACTTC
-              550        560        570        580        590        600

                610        620        630        640        650        660
806559 GATACTGAATTTCGCGATGACCAAACAGAAATGGCTTCGAGGTATCAGCGCTTTGAATAT
       :::::::::::::::::::::::::::::::::::::::: ::::::::::::::::::
       GATACTGAATTTCGCGATGACCAAACAGAAATGGCTTCGATTTATCAGCGCTTTGAATAT
-              610        620        630        640        650        660

                670        680        690        700        710        720
806559 TATCAAGGAATTCGTAGTGATAAAAAAATTACTCCAGATATGCTAGATAAACATTTAGCA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TATCAAGGAATTCGTAGTGATAAAAAAATTACTCCAGATATGCTAGATAAACATTTAGCG
-              670        680        690        700        710        720
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                730       740       750       760       770       780
806559 TTATGGGAAAAATTGGTGTTAACACAACACGCTGATGGTTCAATCACAGGAAAAGCCCTT
       ::::::::::::::: :::::::::::::::::::: :::::::::::::::::::::
_      TTATGGGAAAAATTGGGGTTAACACAACACGCTGATGGCTCAATCACAGGAAAAGCCCTT
                730       740       750       760       770       780

                790       800       810       820       830       840
806559 GATCACCCTAACCGGCAACATTTTATGAAAGTCGAAGGTGTATTTAGTGAGGGGACTCAA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      GATCACCCTAACCGGCAACATTTTATGAAAGTCGAAGGTGTATTTAGTGAGGGGACTCAA
                790       800       810       820       830       840

                850       860       870       880       890       900
806559 AAAGCATTACTTGATGCCAATATGCTAAGAGATGTGGGCAAAACGCTTCTTCAAACTGCT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      AAAGCATTACTTGATGCCAATATGCTAAGAGATGTGGGCAAAACGCTTCTTCAAACTGCT
                850       860       870       880       890       900

                910       920       930       940       950       960
806559 ATTTACTTGCGTAGCGATTCATTATCAGCAACTGATAGAAAAAAATTAGAAGAGCGCTAT
       :::::::::::::::::::::::::::::::: :::::::::::::::::::::::::
_      ATTTACTTGCGTAGCGATTCATTATCAGCAACTGGTAGAAAAAAATTAGAAGAGCGCTAT
                910       920       930       940       950       960

                970       980       990      1000      1010      1020
806559 TTATTAGGTACTCGTTATGTCCTTGAACAAGGTTTTCACCGAGGAAGTGGTTATCAAATT
       :::::::::::::::::::::::::::::::::    ::::::::::::::::::::
_      TTATTAGGTACTCGTTATGTCCTTGAACAAGGTTTTACACGAGGAAGTGGTTATCAAATT
                970       980       990      1000      1010      1020

               1030      1040      1050      1060      1070      1080
806559 ATTAGCCATGTTGGTTACCAAACCAGAGAACTTTTTGATGCATGGTTTATTGGTCGTCAT
       ::::  :::::::::::::::::::::::::::::::::::::::::::::::: ::::::
_      ATTACTCATGTTGGTTACCAAACCAGAGAACTTTTTGATGCATGGTTTATTGGCCGTCAT
               1030      1040      1050      1060      1070      1080

               1090      1100      1110      1120      1130      1140
806559 GTTCTTGCAAAAAATAACCTTTTAGCCCCCACTCAACAAGCTATGATGTGGTACAACGCC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      GTTCTTGCAAAAAATAACCTTTTAGCCCCCACTCAACAAGCTATGATGTGGTACAACGCC
               1090      1100      1110      1120      1130      1140

               1150      1160      1170      1180      1190      1200
806559 ACAGGACGTATTTTTGAAAAAAATAATGAAATTGTTGATGCAAATGTCGATATTCTCAAT
       :::::::::::::::::::: :::::::::::::::::::::::::::::::::::::
_      ACAGGACGTATTTTTGAAAAAGATAATGAAATTGTTGATGCAAATGTCGATATTCTCAAT
               1150      1160      1170      1180      1190      1200

               1210      1220      1230      1240      1250      1260
806559 ACTCAATTGCAATGGATGATAAAAAGCTTATTGATGCTACCGGATTATCAACAACGTCAA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      ACTCAATTGCAATGGATGATAAAAAGCTTATTGATGCTACCGGATTATCAACAACGTCAA
               1210      1220      1230      1240      1250      1260

               1270      1280      1290      1300      1310      1320
806559 CAAGCCTTAGCGCAACTGCAACGTTGGCTAAATAAAACCATTCTAAGCTCAAAAGGTGTT
       :::::::::::::::::::: :::::::::::::::::::::::::::::::::::::
_      CAAGCCTTAGCGCAACTGCAAAGTTGGCTAAATAAAACCATTCTAAGCTCAAAAGGTGTT
               1270      1280      1290      1300      1310      1320

               1330      1340      1350      1360      1370      1380
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806559 GCTGGCGGTTTCAAATCTGATGGTTCTATTTTTCACCATTCACAACATTACCCCGCTTAT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GCTGGCGGTTTCAAATCTGATGGTTCTATTTTTCACCATTCACAACATTACCCCGCTTAT
-            1330      1340      1350      1360      1370      1380

             1390      1400      1410      1420      1430      1440
806559 GCTAAAGATGCATTTGGTGGTTTAGCACCCAGTGTTTATGCATTAAGTGATTCACCTTTT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GCTAAAGATGCATTTGGTGGTTTAGCACCCAGTGTTTATGCATTAAGTGATTCACCTTTT
-            1390      1400      1410      1420      1430      1440

             1450      1460      1470      1480      1490      1500
806559 CGCTTATCTACTTCAGCACATGAGCGTTTAAAAGATGTTTTGTTAAAAATGCGGATCTAC
       :::::::::::::::::::::::: ::::::::::::::::::::::::::::::::::
       CGCTTATCTACTTCAGCACATGAGCATTTAAAAGATGTTTTGTTAAAAATGCGGATCTAC
-            1450      1460      1470      1480      1490      1500

             1510      1520      1530      1540      1550      1560
806559 ACCAAAGAGACACAAATTCCTGCTGTATTAAGTGGTCGTCATCCAACTGGGTTGCATAAA
       :::::::::::::::::::::  ::::::::::::::::::::::::::::::::::::
       ACCAAACAGACACAAATTCCTGTGGTATTAAGTGGTCGTCATCCAACTGGGTTGCATAAA
-            1510      1520      1530      1540      1550      1560

             1570      1580      1590      1600      1610      1620
806559 ATAGGGATCGCGCCATTTAAATGGATGGCATTAGCAGGAACCCCAGATGGCAAACAAAAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       ATAGGGATCGCGCCATTTAAATGGATGGCATTAGCAGGAACCCCAGATGGCAAACAAAAG
-            1570      1580      1590      1600      1610      1620

             1630      1640      1650      1660      1670      1680
806559 TTAGATACCACATTATCCGCCGCTTATGCAAAATTAGACAACAAAACGCATTTTGAAGGC
       :::::::::::::::::::::::::::::: ::;:::::::::::::::::::::::::
       TTAGATACCACATTATCCGCCGCTTATGCAAACTTAGACAACAAAACGCATTTTGAAGGC
-            1630      1640      1650      1660      1670      1680

             1690      1700      1710      1720      1730      1740
806559 ATTAAGGCTGAAAGTGAGCCAGTCGGCGCATGGGCAATGAATTATGCATCAATGGCAATA
       ::::: ::::::::::::::::::::::::::::::::::::::::::::::::::::::
       ATTAACGCTGAAAGTGAGCCAGTCGGCGCATGGGCAATGAATTATGCATCAATGGCAATA
-            1690      1700      1710      1720      1730      1740

             1750      1760      1770      1780      1790      1800
806559 CAACGAAGAGCATCGACCCAATCACCACAACAAAGCTGGCTCGCCATAGCGCGCGGTTTT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       CAACGAAGAGCATCGACCCAATCACCACAACAAAGCTGGCTCGCCATAGCGCGCGGTTTT
-            1750      1760      1770      1780      1790      1800

             1810      1820      1830      1840      1850      1860
806559 AGCCGTTATCTTGTTGGTAATGAAAGCTATGAAAATAACAACCGTTATGGTCGTTATTTA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       AGCCGTTATCTTGTTGGTAATGAAAGCTATGAAAATAACAACCGTTATGGTCGTTATTTA
-            1810      1820      1830      1840      1850      1860

             1870      1880      1890      1900      1910      1920
806559 CAATATGGACAATTGGAAATTATTCCAGCTGATTTAACTCAATCAGGGTTTAGCCATGCT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       CAATATGGACAATTGGAAATTATTCCAGCTGATTTAACTCAATCAGGGTTTAGCCATGCT
-            1870      1880      1890      1900      1910      1920

             1930      1940      1950      1960      1970      1980
806559 GGATGGGATTGGAATAGATATCCAGGTACAACAACTATTCATCTTCCCTATAACGAACTT
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          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          GGATGGGATTGGAATAGATATCCAGGTACAACAACTATTCATCTTCCCTATAACGAACTT
_                 1930       1940       1950       1960       1970       1980

                  1990       2000       2010       2020       2030       2040
806559    GAAGCAAAACTTAATCAATTACCTGCTGCAGGTATTGAAGAAATGTTGCTTTCAACAGAA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          GAAGCAAAACTTAATCAATTACCTGCTGCAGGTATTGAAGAAATGTTGCTTTCAACAGAA
_                 1990       2000       2010       2020       2030       2040

                  2050       2060       2070       2080       2090       2100
806559    AGTTACTCTGGTGCAAATACCCTTAATAATAACAGTATGTTTGCCATGAAATTACACGGT
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          AGTTACTCTGGTGCAAATACCCTTAATAATAACAGTATGTTTGCCATGAAATTACACGGT
_                 2050       2060       2070       2080       2090       2100

                  2110       2120       2130       2140       2150       2160
806559    CCAAGTAAATATCAACAACAAAGCTTAAGGGCAAATAAATCCTATTTCTTATTTGATAAT
          :  ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          CACAGTAAATATCAACAACAAAGCTTAAGGGCAAATAAATCCTATTTCTTATTTGATAAT
_                 2110       2120       2130       2140       2150       2160

                  2170       2180       2190       2200       2210       2220
806559    AGAGTTATTGCTTTAGGCTCAGGTATTGAAAATGATGATAAACAACATACGACCGAAACA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          AGAGTTATTGCTTTAGGCTCAGGTATTGAAAATGATGATAAACAACATACGACCGAAACA
_                 2170       2180       2190       2200       2210       2220

                  2230       2240       2250       2260       2270       2280
806559    ACACTATTCCAGTTTGCCGTCCCTAAATTACAGTCAGTGATCATTAATGGCAAAAAGGTA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          ACACTATTCCAGTTTGCCGTCCCTAAATTACAGTCAGTGATCATTAATGGCAAAAAGGTA
_                 2230       2240       2250       2260       2270       2280

                  2290       2300       2310       2320       2330       2340
806559    AATCAATTAGATACTCAATTAACTTTAAATAATGCAGATACATTAATTGATCCTGCCGGC
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          AATCAATTAGATACTCAATTAACTTTAAATAATGCAGATACATTAATTGATCCTGCCGGC
_                 2290       2300       2310       2320       2330       2340

                  2350       2360       2370       2380       2390       2400
806559    AATTTATATAAGCTCACTAAAGGACAAACTGTAAAATTTAGTTATCAAAAACAACATTCA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          AATTTATATAAGCTCACTAAAGGACAAACTGTAAAATTTAGTTATCAAAAACAACATTCA
_                 2350       2360       2370       2380       2390       2400

                  2410       2420       2430       2440       2450       2460
806559    CTTGATGATAGAAATTCAAAACCAACAGAACAATTATTTGCAACAGCTGTTATTTCTCAT
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          CTTGATGATAGAAATTCAAAACCAACAGAACAATTATTTGCAACAGCTGTTATTTCTCAT
_                 2410       2420       2430       2440       2450       2460

                  2470       2480       2490       2500       2510       2520
806559    GGTAAGGCACCGAGTAATGAAAATTATGAATATGCAATAGCTATCGAAGCACAAAATAAT
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          GGTAAGGCACCGAGTAATGAAAATTATGAATATGCAATAGCTATCGAAGCACAAAATAAT
_                 2470       2480       2490       2500       2510       2520

                  2530       2540       2550       2560       2570       2580
806559    AAAGCTCCCGAATACACAGTATTACAACATAATGATCAGCCCCATGCGGTAAAAGATAAA
          ::::::::: :::::::::::::::::::::::::::::: :::::::::::::::::
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_       AAAGCTCCCAAATACACAGTATTACAACATAATGATCAGCTCCATGCGGTAAAAGATAAA
                2530       2540       2550       2560       2570       2580

                2590       2600       2610       2620       2630
806559 ATAACCCAAGAAGAGGGATATGCTTTTTTTGAAGCCACTAAGTTAAAATCAGCGGATGC
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       ATAACCCAAGAAGAGGGATATGGTTTTTTTGAAGCCACTAAGTTAAAATCAGCGGATGC
                2590       2600       2610       2620       2630       2640

    2640       2650       2660       2670       2680       2690
806559 AACATTATTATCCAGTGATGCGCCGGTTATGGTCATGGCTAAAATACAAAATCAGCAATT
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       AACATTATTATCCAGTGATGCGCCGGTTATGGTCATGGCTAAAATACAAAATCAGCAATT
                2650       2660       2670       2680       2690       2700

    2700       2710       2720       2730       2740       2750
806559 AACATTAAGTATTGTTAATCCTGATTTAAATTTATATCAAGGTAGAGAAAAAGATCAATT
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       AACATTAAGTATTGTTAATCCTGATTTAAATTTATATCAAGGTAGAGAAAAAGATCAATT
                2710       2720       2730       2740       2750       2760

    2760       2770       2780       2790       2800       2810
806559 TGATGATAAAGGTAATCAAATCGAAGTTAGTGTTTATTCTCGTCATTGGCTTACAGCAGA
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       TGATGATAAAGGTAATCAAATCGAAGTTAGTGTTTATTCTCGTCATTGGCTTACAGCAGA
                2770       2780       2790       2800       2810       2820

    2820       2830       2840       2850       2860       2870
806559 ATCGCAATCAACAAATAGTACTATTACCGTAAAAGGAATATGGAAATTAACGACACCTCA
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       ATCGCAATCAACAAATAGTACTATTACCGTAAAAGGAATATGGAAATTAACGACACCTCA
                2830       2840       2850       2860       2870       2880

    2880       2890       2900       2910       2920       2930
806559 ACCCGGTGTTATTATTAAGCACCACAATAACAACACTCTTATTACGACAACAACCATACA
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       ACCCGGTGTTATTATTAAGCACCACAATAACAACACTCTTATTACGACAACAACCATACA
                2890       2900       2910       2920       2930       2940

    2940       2950       2960       2970
806559 GGCAACACCTACTGTTATTAATTTAGTTAAGTAA
       :::::::::::::::::::::::::::::::::
_       GGCAACACCTACTGTTATTAATTTAGTTAAGTAA
                2950       2960       2970
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The above discrepancies, bold text, at the nucleotide level resulted in 98.3% identity at the amino acid level and the substituted residues are marked in bold text in the following.

SEQ ID NO: 27 Present Invention Chondroitinase ABC II protein

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>_ ABC (present invention)                                    990 aa vs.
>_ ABC (mature)                                               990 aa
scoring matrix: , gap penalties: -12/-2
98.3% identity;              Global alignment score: 6393
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                10        20        30        40        50        60
457676 LPTLSHEAFGDIYLFEGELPNILTTSNNNQLSLSKQHAKDGEQSLKWQYQPQATLTLNNI
       :::::::::::::::::::: :::::::::::::::::::::::::::::::::::::::
_      LPTLSHEAFGDIYLFEGELPNTLTTSNNNQLSLSKQHAKDGEQSLKWQYQPQATLTLNNI
                10        20        30        40        50        60

                70        80        90       100       110       120
457676 VNYQDDKNTATPLTFMMWIYNEKPQSSPLTLAFKQNNKIALSFNAELNFTGWRGIAVPFR
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      VNYQDDKNTATPLTFMMWIYNEKPQSSPLTLAFKQNNKIALSFNAELNFTGWRGIAVPFR
                70        80        90       100       110       120

               130       140       150       160       170       180
457676 DMQGSATGQLDQLVITAPNQAGTLFFDQIIMSVPLDNRWAVPDYQTPYVNNAVNTMVSKN
       :::::.::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      DMQGSVTGQLDQLVITAPNQAGTLFFDQIIMSVPLDNRWAVPDYQTPYVNNAVNTMVSKN
               130       140       150       160       170       180

               190       200       210       220       230       240
457676 WSALLMYDQMFQAHYPTLNFDTEFRDDQTEMASIYQRFEYYQGIRSDKKITPDMLDKHLA
       :::::::::::::::::::::::::::::::: :::::::::::::::::::::::::::
_      WSALLMYDQMFQAHYPTLNFDTEFRDDQTEMASRYQRFEYYQGIRSDKKITPDMLDKHLA
               190       200       210       220       230       240

               250       260       270       280       290       300
457676 LWEKLGLTQHADGSITGKALDHPNRQHFMKVEGVFSEGTQKALLDANMLRDVGKTLLQTA
       ::::: ::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      LWEKLVLTQHADGSITGKALDHPNRQHFMKVEGVFSEGTQKALLDANMLRDVGKTLLQTA
               250       260       270       280       290       300

               310       320       330       340       350       360
457676 IYLRSDSLSATGRKKLEERYLLGTRYVLEQGFTRGSGYQIITHVGYQTRELFDAWFIGRH
       :::::::::: :::::::::::::::::::: :::::::.:::::::::::::::::::::
_      IYLRSDSLSATDRKKLEERYLLGTRYVLEQGFHRGSGYQIISHVGYQTRELFDAWFIGRH
               310       320       330       340       350       360

               370       380       390       400       410       420
457676 VLAKNNLLAPTQQAMMWYNATGRIFEKDNEIVDANVDILNTQLQWMIKSLLMLPDYQQRQ
       ::::::::::::::::::::::::::::.:::::::::::::::::::::::::::::::::
_      VLAKNNLLAPTQQAMMWYNATGRIFEKNNEIVDANVDILNTQLQWMIKSLLMLPDYQQRQ
               370       380       390       400       410       420

               430       440       450       460       470       480
457676 QALAQLQSWLNKTILSSKGVAGGFKSDGSIFHHSQHYPAYAKDAFGGLAPSVYALSDSPF
       ::::::: ::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      QALAQLQRWLNKTILSSKGVAGGFKSDGSIFHHSQHYPAYAKDAFGGLAPSVYALSDSPF
               430       440       450       460       470       480

               490       500       510       520       530       540
457676 RLSTSAHEHLKDVLLKMRIYTKETQIPVVLSGRHPTGLHKIGIAPFKWMALAGTPDGKQK
       :::::::::.::::::::::::::::::::.::::::::::::::::::::::::::::::::
_      RLSTSAHERLKDVLLKMRIYTKETQIPAVLSGRHPTGLHKIGIAPFKWMALAGTPDGKQK
               490       500       510       520       530       540

               550       560       570       580       590       600
457676 LDTTLSAAYANLDNKTHFEGINAESEPVGAWAMNYASMAIQRRASTQSPQQSWLAIARGF
       ::::::::::.::::::::::.::::::::::::::::::::::::::::::::::::::::
_      LDTTLSAAYAKLDNKTHFEGIKAESEPVGAWAMNYASMAIQRRASTQSPQQSWLAIARGF
               550       560       570       580       590       600

               610       620       630       640       650       660
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457676 SRYLVGNESYENNNRYGRYLQYGQLEIIPADLTQSGFSHAGWDWNRYPGTTTIHLPYNEL
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       SRYLVGNESYENNNRYGRYLQYGQLEIIPADLTQSGFSHAGWDWNRYPGTTTIHLPYNEL
-             610       620       630       640       650       660

              670       680       690       700       710       720
457676 EAKLNQLPAAGIEEMLLSTESYSGANTLNNNSMFAMKLHGHSKYQQQSLRANKSYFLFDN
       :::::::::::::::::::::::::::::::::::::: :::::::::::::::::::
       EAKLNQLPAAGIEEMLLSTESYSGANTLNNNSMFAMKLHGPSKYQQQSLRANKSYFLFDN
-             670       680       690       700       710       720

              730       740       750       760       770       780
457676 RVIALGSGIENDDKQHTTETTLFQFAVPKLQSVIINGKKVNQLDTQLTLNNADTLIDPAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       RVIALGSGIENDDKQHTTETTLFQFAVPKLQSVIINGKKVNQLDTQLTLNNADTLIDPAG
-             730       740       750       760       770       780

              790       800       810       820       830       840
457676 NLYKLTKGQTVKFSYQKQHSLDDRNSKPTEQLFATAVISHGKAPSNENYEYAIAIEAQNN
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       NLYKLTKGQTVKFSYQKQHSLDDRNSKPTEQLFATAVISHGKAPSNENYEYAIAIEAQNN
-             790       800       810       820       830       840

              850       860       870       880       890       900
457676 KAPKYTVLQHNDQLHAVKDKITQEEGYGFFEATKLKSADATLLSSDAPVMVMAKIQNQQL
       :::.:::::::::: ::::::::::::.::::::::::::::::::::::::::::::::
       KAPEYTVLQHNDQPHAVKDKITQEEGYAFFEATKLKSADATLLSSDAPVMVMAKIQNQQL
-             850       860       870       880       890       900

              910       920       930       940       950       960
457676 TLSIVNPDLNLYQGREKDQFDDKGNQIEVSVYSRHWLTAESQSTNSTITVKGIWKLTTPQ
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TLSIVNPDLNLYQGREKDQFDDKGNQIEVSVYSRHWLTAESQSTNSTITVKGIWKLTTPQ
-             910       920       930       940       950       960

              970       980       990
457676 PGVIIKHHNNNTLITTTTIQATPTVINLVK
       ::::::::::::::::::::::::::::::
       PGVIIKHHNNNTLITTTTIQATPTVINLVK
-             970       980       990
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SEQ ID NO: 28 Present Invention Chondroitinase ABC I nucleic acid

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>_ ABCI present invention                                  2994 nt vs.
>_ ABCI mature                                             2994 nt
scoring matrix: , gap penalties: -12/-2
99.7% identity;          Global alignment score: 11909

               10        20        30        40        50        60
806559 GCCACCAGCAATCCTGCATTTGATCCTAAAAATCTGATGCAGTCAGAAATTTACCATTTT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GCCACCAGCAATCCTGCATTTGATCCTAAAAATCTGATGCAGTCAGAAATTTACCATTTT
-              10        20        30        40        50        60

               70        80        90       100       110       120
806559 GCACAAAATAACCCATTAGCAGACTTCTCATCAGATAAAAACTCAATACTAACGTTATCT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GCACAAAATAACCCATTAGCAGACTTCTCATCAGATAAAAACTCAATACTAACGTTATCT
-              70        80        90       100       110       120
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              130        140        150        160        170        180
806559 GATAAACGTAGCATTATGGGAAACCAATCTCTTTTATGGAAATGGAAAGGTGGTAGTAGC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      GATAAACGTAGCATTATGGGAAACCAATCTCTTTTATGGAAATGGAAAGGTGGTAGTAGC
              130        140        150        160        170        180

              190        200        210        220        230        240
806559 TTTACTTTACATAAAAAACTGATTGTCCCCACCGATAAAGAAGCATCTAAAGCATGGGGA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      TTTACTTTACATAAAAAACTGATTGTCCCCACCGATAAAGAAGCATCTAAAGCATGGGGA
              190        200        210        220        230        240

              250        260        270        280        290        300
806559 CGCTCATCCACCCCCGTTTTCTCATTTTGGCTTTACAATGAAAAACCGATTGATGGTTAT
       :::::::: ::::::::::::::::::::::::::::::::::::::::::::::::::::
_      CGCTCATCTACCCCCGTTTTCTCATTTTGGCTTTACAATGAAAAACCGATTGATGGTTAT
              250        260        270        280        290        300

              310        320        330        340        350        360
806559 CTTACTATCGATTTCGGAGAAAAACTCATTTCAACCAGTGAGGCTCAGGCAGGCTTTAAA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      CTTACTATCGATTTCGGAGAAAAACTCATTTCAACCAGTGAGGCTCAGGCAGGCTTTAAA
              310        320        330        340        350        360

              370        380        390        400        410        420
806559 GTAAAATTAGATTTCACTGGCTGGCGTACTGTGGGAGTCTCTTTAAATAACGATCTTGAA
       :::::::::::::::::::::::::: ::::::::::::::::::::::::::::::::
_      GTAAAATTAGATTTCACTGGCTGGCGTGCTGTGGGAGTCTCTTTAAATAACGATCTTGAA
              370        380        390        400        410        420

              430        440        450        460        470        480
806559 AATCGAGAGATGACCTTAAATGCAACCAATACCTCCTCTGATGGTACTCAAGACAGCATT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      AATCGAGAGATGACCTTAAATGCAACCAATACCTCCTCTGATGGTACTCAAGACAGCATT
              430        440        450        460        470        480

              490        500        510        520        530        540
806559 GGGCGTTCTTTAGGTGCTAAAGTCGATAGTATTCGTTTTAAAGCGCCTTCTAATGTGAGT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      GGGCGTTCTTTAGGTGCTAAAGTCGATAGTATTCGTTTTAAAGCGCCTTCTAATGTGAGT
              490        500        510        520        530        540

              550        560        570        580        590        600
806559 CAGGGTGAAATCTATATCGACCGTATTATGTTTTCTGTCGATGATGCTCGCTACCAATGG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      CAGGGTGAAATCTATATCGACCGTATTATGTTTTCTGTCGATGATGCTCGCTACCAATGG
              550        560        570        580        590        600

              610        620        630        640        650        660
806559 TCTGATTATCAAGTAAAAACTCGCTTATCAGAACCTGAAATTCAATTTCACAACGTAAAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      TCTGATTATCAAGTAAAAACTCGCTTATCAGAACCTGAAATTCAATTTCACAACGTAAAG
              610        620        630        640        650        660

              670        680        690        700        710        720
806559 CCACAACTACCTGTAACACCTGAAAATTTAGCGGCCATTGATCTTATTCGCCAACGTCTA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      CCACAACTACCTGTAACACCTGAAAATTTAGCGGCCATTGATCTTATTCGCCAACGTCTA
              670        680        690        700        710        720
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                730        740        750        760        770        780
806559 ATTAATGAATTTGTCGGAGGTGAAAAAGAGACAAACCTCGCATTAGAAGAGAATATCAGC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       ATTAATGAATTTGTCGGAGGTGAAAAAGAGACAAACCTCGCATTAGAAGAGAATATCAGC
-               730        740        750        760        770        780

                790        800        810        820        830        840
806559 AAATTAAAAAGTGATTTCGATGCTCTTAATACTCACACTTTAGCAAATGGTGGAACGCAA
       :::::::::::::::::::::::::::::: :::::::::::::::::::::::::::::
       AAATTAAAAAGTGATTTCGATGCTCTTAATATTCACACTTTAGCAAATGGTGGAACGCAA
-               790        800        810        820        830        840

                850        860        870        880        890        900
806559 GGCAGACATCTGATCACTGATAAACAAATCATTATTTATCAACCAGAGAATCTTAACTCT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GGCAGACATCTGATCACTGATAAACAAATCATTATTTATCAACCAGAGAATCTTAACTCC
-               850        860        870        880        890        900

                910        920        930        940        950        960
806559 CAAGATAAACAACTATTTGATAATTATGTTATTTTAGGTAATTACACGACATTAATGTTT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       CAAGATAAACAACTATTTGATAATTATGTTATTTTAGGTAATTACACGACATTAATGTTT
-               910        920        930        940        950        960

                970        980        990       1000       1010       1020
806559 AATATTAGCCGTGCTTATGTGCTGGAAAAAGATCCCACACAAAAGGCGCAACTAAAGCAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       AATATTAGCCGTGCTTATGTGCTGGAAAAAGATCCCACACAAAAGGCGCAACTAAAGCAG
-               970        980        990       1000       1010       1020

               1030       1040       1050       1060       1070       1080
806559 ATGTACTTATTAATGACAAAGCATTTATTAGATCAAGGCTTTGTTAAAGGGAGTGCTTTA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       ATGTACTTATTAATGACAAAGCATTTATTAGATCAAGGCTTTGTTAAAGGGAGTGCTTTA
-               1030       1040       1050       1060       1070       1080

               1090       1100       1110       1120       1130       1140
806559 GTGACAACCCATCACTGGGGATACAGTTCTCGTTGGTGGTATATTTCCACGTTATTAATG
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GTGACAACCCATCACTGGGGATACAGTTCTCGTTGGTGGTATATTTCCACGTTATTAATG
-               1090       1100       1110       1120       1130       1140

               1150       1160       1170       1180       1190       1200
806559 TCTGATGCACTAAAAGAAGCGAACCTACAAACTCAAGTTTATGATTCATTACTGTGGTAT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TCTGATGCACTAAAAGAAGCGAACCTACAAACTCAAGTTTATGATTCATTACTGTGGTAT
-               1150       1160       1170       1180       1190       1200

               1210       1220       1230       1240       1250       1260
806559 TCACGTGAGTTTAAAAGTAGTTTTGATATGAAAGTAAGTGCTGATAGCTCTGATCTAGAT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TCACGTGAGTTTAAAAGTAGTTTTGATATGAAAGTAAGTGCTGATAGCTCTGATCTAGAT
-               1210       1220       1230       1240       1250       1260

               1270       1280       1290       1300       1310       1320
806559 TATTTCAATACCTTATCTCGCCAACATTTAGCCTTATTACTACTAGAGCCTGATGATCAA
       :::::::::::::::::::::::::::::::::::: :::::::::::::::::::
       TATTTCAATACCTTATCTCGCCAACATTTAGCCTTATTATTACTAGAGCCTGATGATCAA
-               1270       1280       1290       1300       1310       1320

               1330       1340       1350       1360       1370       1380
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806559 AAGCGTATCAACTTAGTTAATACTTTCAGCCATTATATCACTGGCGCATTAACGCAAGTG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       AAGCGTATCAACTTAGTTAATACTTTCAGCCATTATATCACTGGCGCATTAACGCAAGTG
_           1330       1340       1350       1360       1370       1380

            1390       1400       1410       1420       1430       1440
806559 CCACCGGGTGGTAAAGATGGTTTACGCCCTGATGGTACAGCATGGCGACATGAAGGCAAC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       CCACCGGGTGGTAAAGATGGTTTACGCCCTGATGGTACAGCATGGCGACATGAAGGCAAC
_           1390       1400       1410       1420       1430       1440

            1450       1460       1470       1480       1490       1500
806559 TATCCGGGCTACTCTTTCCCAGCCTTTAAAAATGCCTCTCAGCTTATTTATTTATTACGC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TATCCGGGCTACTCTTTCCCAGCCTTTAAAAATGCCTCTCAGCTTATTTATTTATTACGC
_           1450       1460       1470       1480       1490       1500

            1510       1520       1530       1540       1550       1560
806559 GATACACCATTTTCAGTGGGTGAAAGTGGTTGGAATAGCCTGAAAAAAGCGATGGTTTCA
       :::::::::::::::::::::::::::::::::: :::::::::::::::::::::::::
       GATACACCATTTTCAGTGGGTGAAAGTGGTTGGAATAACCTGAAAAAAGCGATGGTTTCA
_           1510       1520       1530       1540       1550       1560

            1570       1580       1590       1600       1610       1620
806559 GCGTGGATCTACAGTAATCCAGAAGTTGGATTACCGCTTGCAGGAAGACACCCTCTTAAC
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GCGTGGATCTACAGTAATCCAGAAGTTGGATTACCGCTTGCAGGAAGACACCCTTTTAAC
_           1570       1580       1590       1600       1610       1620

            1630       1640       1650       1660       1670       1680
806559 TCACCTTCGTTAAAATCAGTCGCTCAAGGCTATTACTGGCTTGCCATGTCTGCAAAATCA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TCACCTTCGTTAAAATCAGTCGCTCAAGGCTATTACTGGCTTGCCATGTCTGCAAAATCA
_           1630       1640       1650       1660       1670       1680

            1690       1700       1710       1720       1730       1740
806559 TCGCCTGATAAAACACTTGCATCTATTTATCTTGCGATTAGTGATAAAACACAAAATGAA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TCGCCTGATAAAACACTTGCATCTATTTATCTTGCGATTAGTGATAAAACACAAAATGAA
_           1690       1700       1710       1720       1730       1740

            1750       1760       1770       1780       1790       1800
806559 TCAACTGCTATTTTTGGAGAAACTATTACACCAGCGTCTTTACCTCAAGGTTTCTATGCC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TCAACTGCTATTTTTGGAGAAACTATTACACCAGCGTCTTTACCTCAAGGTTTCTATGCC
_           1750       1760       1770       1780       1790       1800

            1810       1820       1830       1840       1850       1860
806559 TTTAATGGCGGTGCTTTTGGTATTCATCGTTGGCAAGATAAAATGGTGACACTGAAAGCT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TTTAATGGCGGTGCTTTTGGTATTCATCGTTGGCAAGATAAAATGGTGACACTGAAAGCT
_           1810       1820       1830       1840       1850       1860

            1870       1880       1890       1900       1910       1920
806559 TATAACACCAATGTTTGGTCATCTGAAATTTATAACAAAGATAACCGTTATGGCCGTTAC
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TATAACACCAATGTTTGGTCATCTGAAATTTATAACAAAGATAACCGTTATGGCCGTTAC
_           1870       1880       1890       1900       1910       1920

            1930       1940       1950       1960       1970       1980
806559 CAAAGTCATGGTGTCGCTCAAATAGTGAGTAATGGCTCGCAGCTTTCACAGGGCTATCAG
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          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          CAAAGTCATGGTGTCGCTCAAATAGTGAGTAATGGCTCGCAGCTTTCACAGGGCTATCAG
_                 1930      1940      1950      1960      1970      1980

                  1990      2000      2010      2020      2030      2040
806559    CAAGAAGGTTGGGATTGGAATAGAATGCCAGGGGCAACCACTATCCACCTTCCTCTTAAA
          :::::::::::::::::::::::::: :::::::::::::::: ::::::::::::::::
          CAAGAAGGTTGGGATTGGAATAGAATGCAAGGGGCAACCACTATTCACCTTCCTCTTAAA
_                 1990      2000      2010      2020      2030      2040

                  2050      2060      2070      2080      2090      2100
806559    GACTTAGACAGTCCTAAACCTCATACCTTAATGCAACGTGGAGAGCGTGGATTTAGCGGA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          GACTTAGACAGTCCTAAACCTCATACCTTAATGCAACGTGGAGAGCGTGGATTTAGCGGA
_                 2050      2060      2070      2080      2090      2100

                  2110      2120      2130      2140      2150      2160
806559    ACATCATCCCTTGAAGGTCAATATGGCATGATGGCATTCGATCTTATTTATCCCGCCAAT
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          ACATCATCCCTTGAAGGTCAATATGGCATGATGGCATTCGATCTTATTTATCCCGCCAAT
_                 2110      2120      2130      2140      2150      2160

                  2170      2180      2190      2200      2210      2220
806559    CTTGAGCGTTTTGATCCTAATTTCACTGCGAAAAAGAGTGTATTAGCCGCTGATAATCAC
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          CTTGAGCGTTTTGATCCTAATTTCACTGCGAAAAAGAGTGTATTAGCCGCTGATAATCAC
_                 2170      2180      2190      2200      2210      2220

                  2230      2240      2250      2260      2270      2280
806559    TTAATTTTTATTGGTAGCAATATAAATAGTAGTGATAAAAATAAAAATGTTGAAACGACC
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          TTAATTTTTATTGGTAGCAATATAAATAGTAGTGATAAAAATAAAAATGTTGAAACGACC
_                 2230      2240      2250      2260      2270      2280

                  2290      2300      2310      2320      2330      2340
806559    TTATTCCAACATGCCATTACTCCAACATTAAATACCCTTTGGATTAATGGACAAAAGATA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          TTATTCCAACATGCCATTACTCCAACATTAAATACCCTTTGGATTAATGGACAAAAGATA
_                 2290      2300      2310      2320      2330      2340

                  2350      2360      2370      2380      2390      2400
806559    GAAAACATGCCTTATCAAACAACACTTCAACAAGGTGATTGGTTAATTGATAGCAATGGC
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          GAAAACATGCCTTATCAAACAACACTTCAACAAGGTGATTGGTTAATTGATAGCAATGGC
_                 2350      2360      2370      2380      2390      2400

                  2410      2420      2430      2440      2450      2460
806559    AATGGTTACTTAATTACTCAAGCAGAAAAAGTAAATGTAAGTCGCCAACATCAGGTTTCA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          AATGGTTACTTAATTACTCAAGCAGAAAAAGTAAATGTAAGTCGCCAACATCAGGTTTCA
_                 2410      2420      2430      2440      2450      2460

                  2470      2480      2490      2500      2510      2520
806559    GCGGAAAATAAAAATCGCCAACCGACAGAAGGAAACTTTAGCTCGGCATGGATCGATCAC
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          GCGGAAAATAAAAATCGCCAACCGACAGAAGGAAACTTTAGCTCGGCATGGATCGATCAC
_                 2470      2480      2490      2500      2510      2520

                  2530      2540      2550      2560      2570      2580
806559    AGCACTCGCCCCAAAGATGCCAGTTATGAGTATATGGTCTTTTTAGATGCGACACCTGAA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
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_       AGCACTCGCCCCAAAGATGCCAGTTATGAGTATATGGTCTTTTTAGATGCGACACCTGAA
                2530       2540       2550       2560       2570       2580

                2590       2600       2610       2620       2630       2640
806559  AAAATGGGAGAGATGGCACAAAAATTCCGTGAAAATAATGGGTTATATCAGGTTCTTCGT
        :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       AAAATGGGAGAGATGGCACAAAAATTCCGTGAAAATAATGGGTTATATCAGGTTCTTCGT
                2590       2600       2610       2620       2630       2640

                2650       2660       2670       2680       2690       2700
806559  AAGGATAAAGACGTTCATATTATTCTCGATAAACTCAGCAATGTAACGGGATATGCCTTT
        :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       AAGGATAAAGACGTTCATATTATTCTCGATAAACTCAGCAATGTAACGGGATATGCCTTT
                2650       2660       2670       2680       2690       2700

                2710       2720       2730       2740       2750       2760
806559  TATCAGCCAGCATCAATTGAAGACAAATGGATCAAAAAGGTTAATAAACCTGCAATTGTG
        :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       TATCAGCCAGCATCAATTGAAGACAAATGGATCAAAAAGGTTAATAAACCTGCAATTGTG
                2710       2720       2730       2740       2750       2760

                2770       2780       2790       2800       2810       2820
806559  ATGACTCATCGACAAAAAGACACTCTTATTGTCAGTGCAGTTACACCTGATTTAAATATG
        :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       ATGACTCATCGACAAAAAGACACTCTTATTGTCAGTGCAGTTACACCTGATTTAAATATG
                2770       2780       2790       2800       2810       2820

                2830       2840       2850       2860       2870       2880
806559  ACTCGCCAAAAAGCAGCAACTCCTGTCACCATCAATGTCACGATTAATGGCAAATGGCAA
        :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       ACTCGCCAAAAAGCAGCAACTCCTGTCACCATCAATGTCACGATTAATGGCAAATGGCAA
                2830       2840       2850       2860       2870       2880

                2890       2900       2910       2920       2930       2940
806559  TCTGCTGATAAAAATAGTGAAGTGAAATATCAGGTTTCTGGTGATAACACTGAACTGACG
        :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       TCTGCTGATAAAAATAGTGAAGTGAAATATCAGGTTTCTGGTGATAACACTGAACTGACG
                2890       2900       2910       2920       2930       2940

                2950       2960       2970       2980       2990
806559  TTTACGAGTTACTTTGGTATTCCACAAGAAATCAAACTCTCGCCACTCCCTTGA
        :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_       TTTACGAGTTACTTTGGTATTCCACAAGAAATCAAACTCTCGCCACTCCCTTGA
      2950   2960   2970   2980   2990
```

The sequence identity at the amino acid level is shown below:

SEQ ID NO: 29 Present Invention Chondroitinase ABC I protein

>_ ABCI Present invention 997 aa vs.

>_ ABCI mature 997 aa

scoring matrix: , gap penalties: -12/-2

99.5% identity; Global alignment score: 6595

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          10        20        30        40        50        60
365019 ATSNPAFDPKNLMQSEIYHFAQNNPLADFSSDKNSILTLSDKRSIMGNQSLLWKWKGGSS
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      ATSNPAFDPKNLMQSEIYHFAQNNPLADFSSDKNSILTLSDKRSIMGNQSLLWKWKGGSS
          10        20        30        40        50        60

          70        80        90       100       110       120
365019 FTLHKKLIVPTDKEASKAWGRSSTPVFSFWLYNEKPIDGYLTIDFGEKLISTSEAQAGFK
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      FTLHKKLIVPTDKEASKAWGRSSTPVFSFWLYNEKPIDGYLTIDFGEKLISTSEAQAGFK
          70        80        90       100       110       120

         130       140       150       160       170       180
365019 VKLDFTGWRTVGVSLNNDLENREMTLNATNTSSDGTQDSIGRSLGAKVDSIRFKAPSNVS
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      VKLDFTGWRAVGVSLNNDLENREMTLNATNTSSDGTQDSIGRSLGAKVDSIRFKAPSNVS
         130       140       150       160       170       180

         190       200       210       220       230       240
365019 QGEIYIDRIMFSVDDARYQWSDYQVKTRLSEPEIQFHNVKPQLPVTPENLAAIDLIRQRL
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      QGEIYIDRIMFSVDDARYQWSDYQVKTRLSEPEIQFHNVKPQLPVTPENLAAIDLIRQRL
         190       200       210       220       230       240

         250       260       270       280       290       300
365019 INEFVGGEKETNLALEENISKLKSDFDALNTHTLANGGTQGRHLITDKQIIIYQPENLNS
       :::::::::::::::::::::::::::::: ::::::::::::::::::::::::::::::
_      INEFVGGEKETNLALEENISKLKSDFDALNIHTLANGGTQGRHLITDKQIIIYQPENLNS
         250       260       270       280       290       300

         310       320       330       340       350       360
365019 QDKQLFDNYVILGNYTTLMFNISRAYVLEKDPTQKAQLKQMYLLMTKHLLDQGFVKGSAL
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      QDKQLFDNYVILGNYTTLMFNISRAYVLEKDPTQKAQLKQMYLLMTKHLLDQGFVKGSAL
         310       320       330       340       350       360

         370       380       390       400       410       420
365019 VTTHHWGYSSRWWYISTLLMSDALKEANLQTQVYDSLLWYSREFKSSFDMKVSADSSDLD
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      VTTHHWGYSSRWWYISTLLMSDALKEANLQTQVYDSLLWYSREFKSSFDMKVSADSSDLD
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370 380 390 400 410 420

430 440 450 460 470 480

365019 YFNTLSRQHLALLLLEPDDQKRINLVNTFSHYITGALTQVPPGGKDGLRPDGTAWRHEGN

:::

_ YFNTLSRQHLALLLLEPDDQKRINLVNTFSHYITGALTQVPPGGKDGLRPDGTAWRHEGN

430 440 450 460 470 480

490 500 510 520 530 540

365019 YPGYSFPAFKNASQLIYLLRDTPFSVGESGWNSLKKAMVSAWIYSNPEVGLPLAGRHPLN

:::.:

_ YPGYSFPAFKNASQLIYLLRDTPFSVGESGWNNLKKAMVSAWIYSNPEVGLPLAGRHPFN

490 500 510 520 530 540

550 560 570 580 590 600

365019 SPSLKSVAQGYYWLAMSAKSSPDKTLASIYLAISDKTQNESTAIFGETITPASLPQGFYA

:::

_ SPSLKSVAQGYYWLAMSAKSSPDKTLASIYLAISDKTQNESTAIFGETITPASLPQGFYA

550 560 570 580 590 600

610 620 630 640 650 660

365019 FNGGAFGIHRWQDKMVTLKAYNTNVWSSEIYNKDNRYGRYQSHGVAQIVSNGSQLSQGYQ

:::

_ FNGGAFGIHRWQDKMVTLKAYNTNVWSSEIYNKDNRYGRYQSHGVAQIVSNGSQLSQGYQ

610 620 630 640 650 660

670 680 690 700 710 720

365019 QEGWDWNRMPGATTIHLPLKDLDSPKPHTLMQRGERGFSGTSSLEGQYGMMAFDLIYPAN

::::::::: ::

_ QEGWDWNRMQGATTIHLPLKDLDSPKPHTLMQRGERGFSGTSSLEGQYGMMAFDLIYPAN

670 680 690 700 710 720

730 740 750 760 770 780

365019 LERFDPNFTAKKSVLAADNHLIFIGSNINSSDKNKNVETTLFQHAITPTLNTLWINGQKI

:::

_ LERFDPNFTAKKSVLAADNHLIFIGSNINSSDKNKNVETTLFQHAITPTLNTLWINGQKI

730 740 750 760 770 780

790 800 810 820 830 840

365019 ENMPYQTTLQQGDWLIDSNGNGYLITQAEKVNVSRQHQVSAENKNRQPTEGNFSSAWIDH

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     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_    ENMPYQTTLQQGDWLIDSNGNGYLITQAEKVNVSRQHQVSAENKNRQPTEGNFSSAWIDH
          790       800       810       820       830       840

          850       860       870       880       890       900
365019 STRPKDASYEYMVFLDATPEKMGEMAQKFRENNGLYQVLRKDKDVHIILDKLSNVTGYAF
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_    STRPKDASYEYMVFLDATPEKMGEMAQKFRENNGLYQVLRKDKDVHIILDKLSNVTGYAF
          850       860       870       880       890       900

          910       920       930       940       950       960
365019 YQPASIEDKWIKKVNKPAIVMTHRQKDTLIVSAVTPDLNMTRQKAATPVTINVTINGKWQ
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_    YQPASIEDKWIKKVNKPAIVMTHRQKDTLIVSAVTPDLNMTRQKAATPVTINVTINGKWQ
          910       920       930       940       950       960

          970       980       990
365019 SADKNSEVKYQVSGDNTELTFTSYFGIPQEIKLSPLP
     ::::::::::::::::::::::::::::::::::::
_    SADKNSEVKYQVSGDNTELTFTSYFGIPQEIKLSPLP
          970       980       990
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REFERENCES

1. Fethiere J, Eggimann B, Cygler M (1999) Crystal structure of chondroitin AC lyase, a representative of a family of glycosaminoglycan degrading enzymes. J Mol Biol. 288:635-47.

2. Pojasek K, Shriver Z, Kiley, P Venkataraman G and Sasisekharan R. (2001) Biochem Biophys Res Commun. 286:343-51.

3. Huang W, Matte A, Li Y, Kim YS, Linhardt RJ, Su H, Cygler M. (1999) Crystal structure of chondroitinase B from Flavobacterium heparinum and its complex with a disaccharide product at 1.7 A resolution. J Mol Biol. 294:1257-69.

4. Miura RO, Yamagata S, Miura Y, Harada T and Yamagata T. (1995) Anal Biochem. 225:333-40.

5. Yamagata T, Saito H, Habuchi O and Suzuki S. (1968) J Biol Chem. 243:1536-42.

[0089] Although the present invention has been described in considerable detail with reference to certain preferred embodiments thereof, other versions are possible. Therefore the spirit and scope of the appended claims should not be limited to the description and the preferred versions contain within this specification.

CLAIMS:

What is claimed is:

1. An composition comprising:

an isolated nucleic acid comprising a sequence that encodes for mutant proteoglycan degrading polypeptide.

2. The composition of claim 1 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**, Chondroitinase ABC Type II, **(SEQ ID NO: 27)**, Chondroitinase AC, **(SEQ ID NO: 5)**, and Chondroitinase B, **(SEQ ID NO: 12)**, hyaluronidase 1, **(SEQ ID NO: 30)**, hyaluronidase 2, **(SEQ ID NO: 31)**, hyaluronidase 3, **(SEQ ID NO: 32)**, or hyaluronidase 4, **(SEQ ID NO: 33)**.

3. The composition of claim 1 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**, Chondroitinase ABC Type II, **(SEQ ID NO: 27)**, Chondroitinase AC, **(SEQ ID NO: 5)**, and Chondroitinase B, **(SEQ ID NO: 12)**

4. The composition of claim 1 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**.

5. The composition of claim 1 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of Chondroitinase ABC Type II, **(SEQ ID NO: 27)**.

6. The composition of claim 1 wherein the sequence of said nucleic acid is at least 80%, identical to a nucleic acid sequence encoding for a mutant proteoglycan degrading polypeptide.

7. The composition of claim 1 wherein the nucleic acid encodes for a polypeptide that degrades a proteoclycan in a tissue of the central nervous system.

8. The composition of claim 1 wherein the nucleic acid encodes for a polypeptide that degrades a chondroitin sulfate proteoglycan.

9. An expression vector comprising the nucleic acid of claim 1 operably linked to an expression control sequence.

10. The composition of claim 1 further including cells.

11. An composition comprising:

an isolated nucleic acid consisting of a sequence a sequence that encodes for a biologically active mutant proteoglycan degrading polypeptide.

12. The composition of claim 11 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**, Chondroitinase ABC Type II, **(SEQ ID NO: 27)**, Chondroitinase AC, **(SEQ ID NO: 5)**, and Chondroitinase B, **(SEQ ID NO: 12)**, hyaluronidase 1, **(SEQ ID NO: 30)**, hyaluronidase 2, **(SEQ ID NO: 31)**, hyaluronidase 3, **(SEQ ID NO: 32)**, or hyaluronidase 4, **(SEQ ID NO: 33).**

13. The composition of claim 11 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**, Chondroitinase ABC Type II, **(SEQ ID NO: 27)**, Chondroitinase AC, **(SEQ ID NO: 5)**, and Chondroitinase B, **(SEQ ID NO: 12)**

14. The composition of claim 11 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**

15. The composition of claim 11 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of Chondroitinase ABC Type II, **(SEQ ID NO: 27)**.

16. The composition of claim 11 wherein the nucleic acid encodes for a polypeptide that degrades a proteoclycan in a tissue of the central nervous system.

17. The composition of claim 11 wherein the nucleic acid encodes polypeptide that degrades chondroitin sulfate proteoglycan.

18. The composition of claim 11 further including cells.

19. A composition comprising:

a polypeptide comprising the amino acid sequence of a biologically active mutant proteoglycan degrading polypeptide.

20. The composition of claim 19 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**, Chondroitinase ABC Type II, **(SEQ ID NO: 27)**, Chondroitinase AC, **(SEQ ID NO: 5)**, and Chondroitinase B, **(SEQ ID NO: 12)**, hyaluronidase 1, **(SEQ ID NO: 30)**, hyaluronidase 2, **(SEQ ID NO: 31)**, hyaluronidase 3, **(SEQ ID NO: 32)**, or hyaluronidase 4, **(SEQ ID NO: 33)**.

21. The composition of claim 19 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**, Chondroitinase ABC Type II, **(SEQ ID NO: 27)**, Chondroitinase AC, **(SEQ ID NO: 5)**, or Chondroitinase B, **SEQ ID NO: 12)**.

22. The composition of claim 19 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**.

23. The composition of claim 19 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 27)**.

24. The composition of claim 19 proteoglycan degrading polypeptide is **(SEQ ID NO: 2)**, **(SEQ ID NO: 3)**, or **(SEQ ID NO: 4)**.

25. The composition of claim 19 wherein the polypeptide degrades a proteoclycan in a tissue of the central nervous system.

26. The composition of claim 19 wherein the polypeptide degrades a chondroitin sulfate proteoglycan.

27. The composition of claim 19 further including cells.

28. The composition of claim 19 and a pharmaceutically acceptable excipient.

29. The composition of claim 19 further including molecules which block the action of neurite growth inhibitors, molecules which promote neurite adhesion, diagnostic molecules or a combination of these.

30. A method of treating a tissue, the method comprising:

administering a mutant proteoglycan degrading polypeptide composition to the tissue, said tissue including proteoglycan molecules, said composition degrading at least a portion of the proteoglycan in the tissue.

32. The method of claim 30 wherein the tissue is from the CNS.

33. The method of claim 30 wherein the proteoglycan degradation promotes diffusion of molecules into the tissue.

34. The method of claim 30 further comprising the act of identifying tissue from a contusive spinal cord injury.

35. The method of claim 30 wherein the compostion promote neurite regeneration.

36. The method of claim 30 wherein the composition further includes molecules which block the action of neurite growth inhibitors, molecules which promote neurite adhesion, diagnostic molecules or a combination of these.

37. The method of claim 30 wherein the plasticity of the of the nervous system is improved.

38. The method of claim 30 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**, Chondroitinase ABC Type II, **(SEQ ID NO: 27)**, Chondroitinase AC, **(SEQ ID NO: 5)**, and Chondroitinase B, **(SEQ ID NO: 12)**, hyaluronidase 1, **(SEQ ID NO: 30)**, hyaluronidase 2, **(SEQ ID NO: 31)**, hyaluronidase 3, **(SEQ ID NO: 32)**, or hyaluronidase 4, **(SEQ ID NO: 33)**.

39. The method of claim 30 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**, Chondroitinase ABC Type II, **(SEQ ID NO: 27)**, Chondroitinase AC, **(SEQ ID NO: 5)**, or Chondroitinase B, **SEQ ID NO: 12)**.

40. The method of claim 30 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 1)**.

41. The method of claim 30 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, **(SEQ ID NO: 27)**.

42. The method of claim 30 proteoglycan degrading polypeptide is **(SEQ ID NO: 2)**, **(SEQ ID NO: 3)**, or **(SEQ ID NO: 4)**.

43. A purified chondroitinase mutant polypeptide that degrades a proteoglycan.

Anti-His-tag Western blot (top) and Zymogram (bottom) demonstrating Chondroitinase B deletion NΔ120 CΔ120 mutant expression and activity

(A)

Anti-His-tag Western blot (top) and Zymogram (bottom) demonstrating Chondroitinase AC deletion NΔ50 CΔ275 mutant expression and activity

(B)

FIG. 1

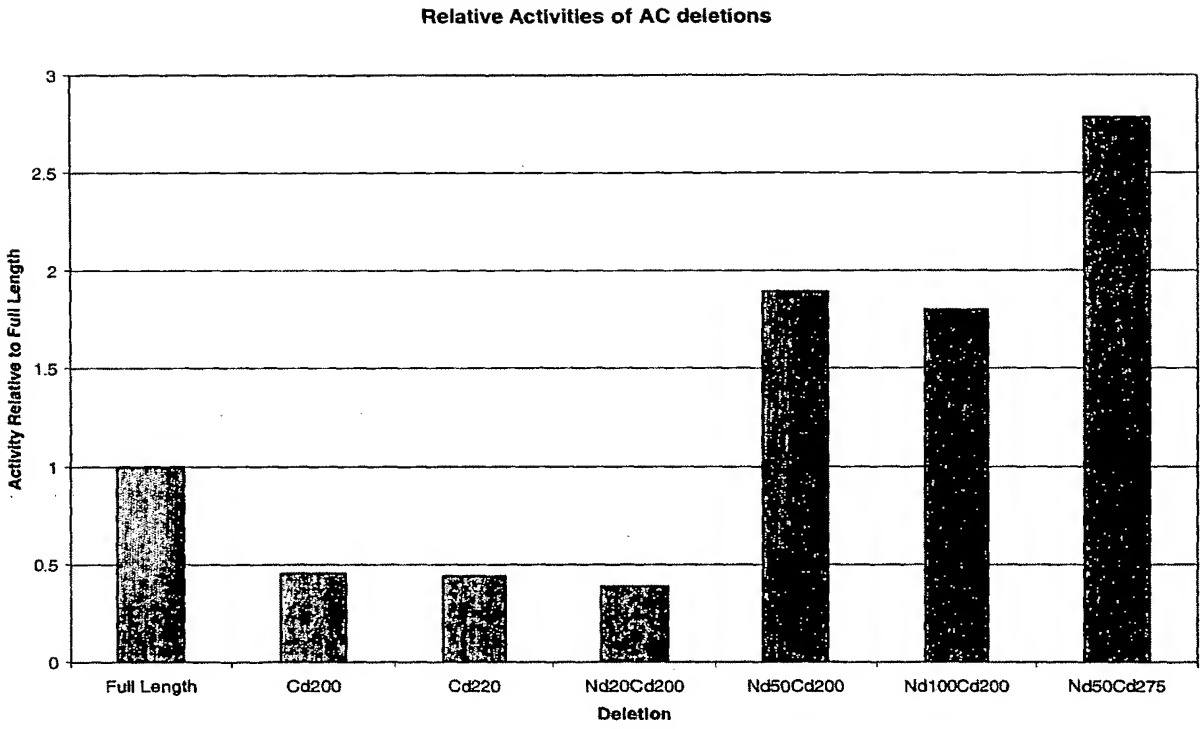
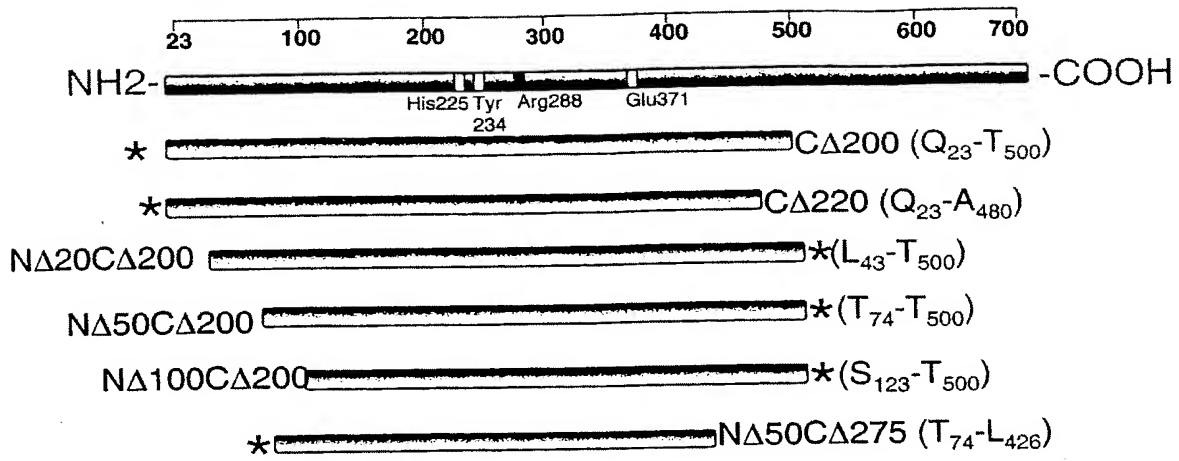
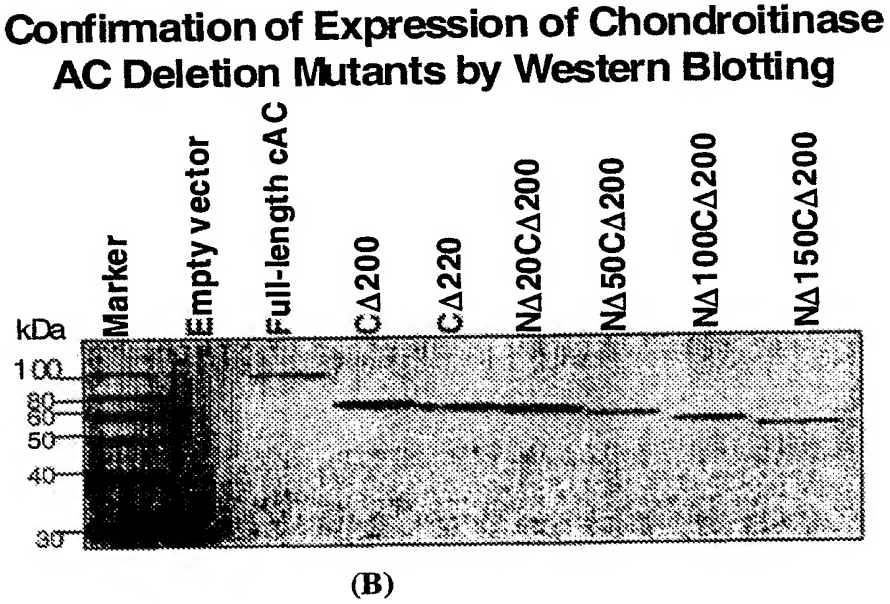


FIG. 2



(A)



(B)

FIG. 3

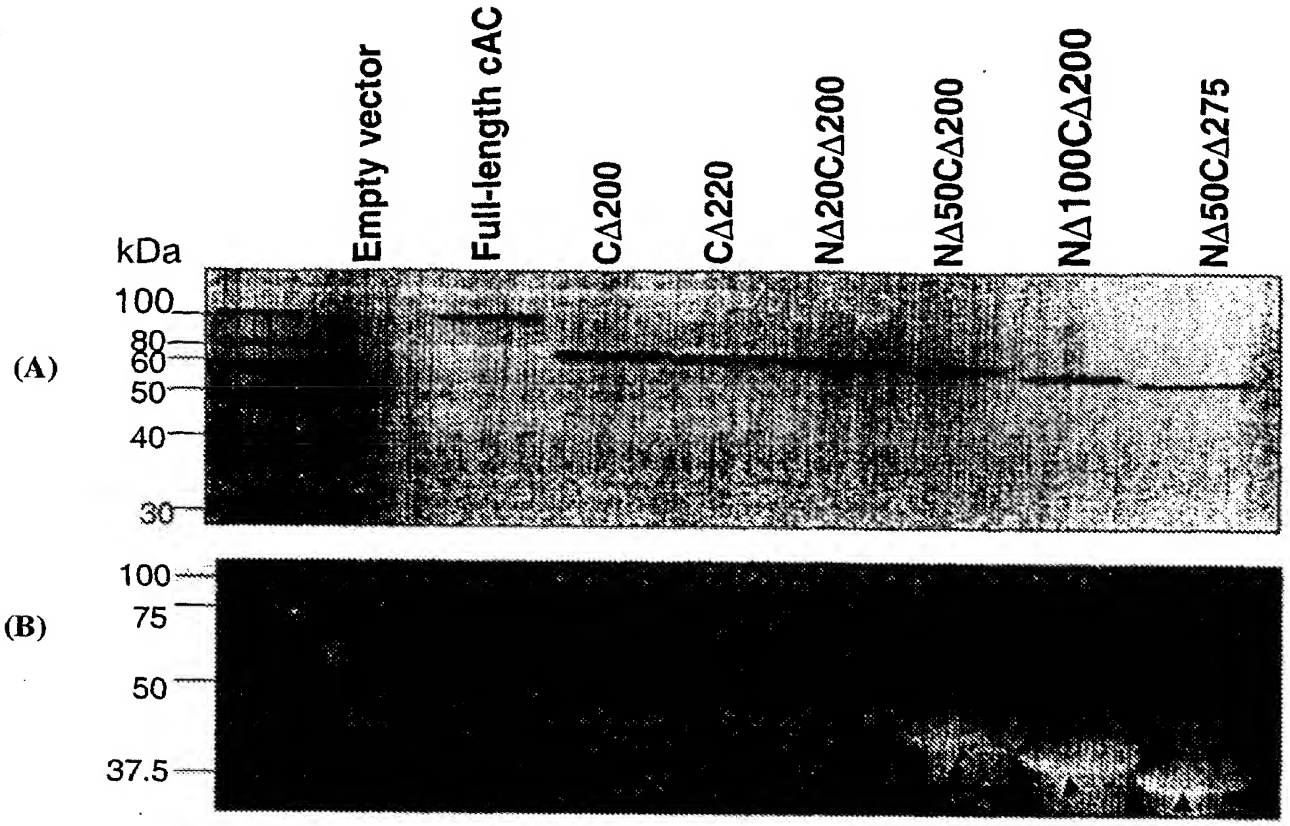


FIG. 4

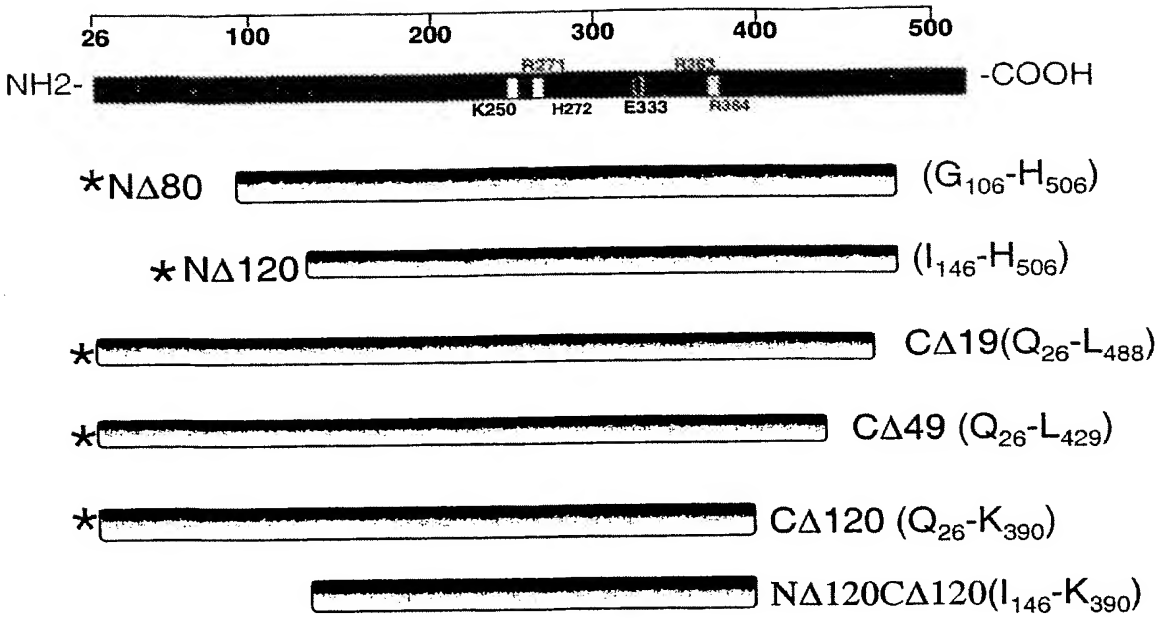


FIG. 5

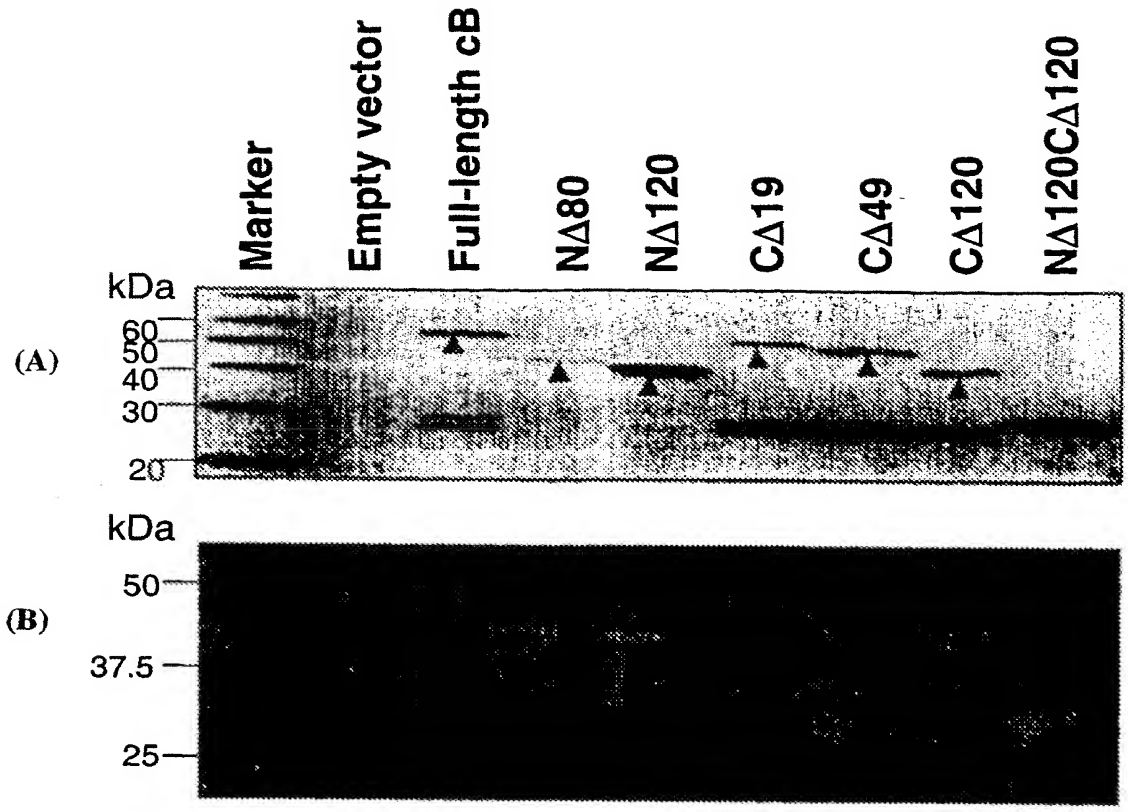


FIG. 6

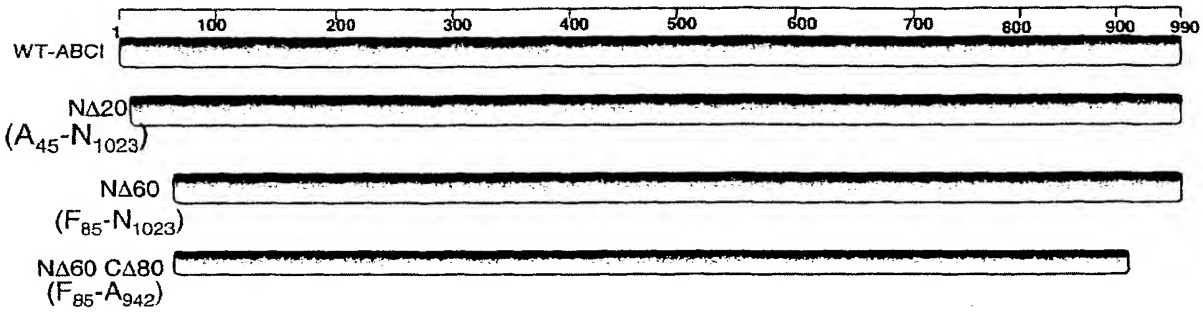


FIG. 7

SEQUENCE LISTING

SEQ ID NO: 1 Chondroitinase ABC I protein

ORIGIN

atsnpa fdpknlmqse iyhfaqnnpl adfssdknsi
61 ltlsdkrsim gnqsllwkwk ggssftlhkk livptdkeas kawgrsstpv fsfwlynekp
121 idgyltidfg eklistseaq agfkvkldft gwrTvgvsln ndlenremtl natntssdgt
181 qdsigrslga kvdsirfkap snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnThtlan
301 ggtqgrhlit dkqiiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka
361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tllmsdalke anlqtqvyds
421 llwysrefks sfdmkvsads sdldyfntls rqhlalllle pddqkrinlv ntfshyitga
481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk
541 amvsawiysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk
601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr
661 ygryqshgva qivsngsqls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
781 vettlfqhai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq
841 hqvsaenknr qptegnfssa widhstrpkd asyeymvfld atpekmgema qkfrenngly
901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk paivmthrqk dtlivsavtp
961 dlnmtrqkaa tpvtinvtin gkwqsadkns evkyqvsgdn teltftsyfg ipqeiklspl
1021 p

SEQ ID NO: 2 NΔ20 ABCI (A_{45}-N_{1023}), protein

aqnnpl adfssdknsi
61 ltlsdkrsim gnqsllwkwk ggssftlhkk livptdkeas kawgrsstpv fsfwlynekp
121 idgyltidfg eklistseaq agfkvkldft gwrtvgvsln ndlenremtl natntssdgt
181 qdsigrslga kvdsirfkap snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnthtlan
301 ggtqgrhlit dkqiiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka
361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tllmsdalke anlqtqvyds
421 llwysrefks sfdmkvsads sdldyfntls rqhlalllle pddqkrinlv ntfshyitga
481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk
541 amvsawiysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk
601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr
661 ygryqshgva qivsngsqls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
781 vettlfqhai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq
841 hqvsaenknr qptegnfssa widhstrpkd asyeymvfld atpekmgema qkfrenngly
901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk paivmthrqk dtlivsavtp
961 dlnmtrqkaa tpvtinvtin gkwqsadkns evkyqvsgdn teltftsyfg ipqeiklspl
1021 p

SEQ ID NO: 3 NΔ60 ABCI (F_{85}-N_{1023}) protein

ftlhkk livptdkeas kawgrsstpv fsfwlynekp
121 idgyltidfg eklistseaq agfkvkldft gwrtvgvsln ndlenremtl natntssdgt
181 qdsigrslga kvdsirfkap snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnthtlan
301 ggtqgrhlit dkqiiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka
361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tllmsdalke anlqtqvyds
421 llwysrefks sfdmkvsads sdldyfntls rqhlalllle pddqkrinlv ntfshyitga
481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk
541 amvsawiysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk
601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr
661 ygryqshgva qivsngsqls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
781 vettlfqhai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq
841 hqvsaenknr qptegnfssa widhstrpkd asyeymvfld atpekmgema qkfrenngly
901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk paivmthrqk dtlivsavtp
961 dlnmtrqkaa tpvtinvtin gkwqsadkns evkyqvsgdn teltftsyfg ipqeiklspl
1021 p

SEQ ID No. 4: NΔ60 CΔ80 ABCI (F_{85}-A_{942}) protein

ftlhkk livptdkeas kawgrsstpv fsfwlynekp
121 idgyltidfg eklistseaq agfkvkldft gwrtvgvsln ndlenremtl natntssdgt
181 qdsigrslga kvdsirfkap snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnthtlan
301 ggtqgrhlit dkqiiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka
361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tllmsdalke anlqtqvyds
421 llwysrefks sfdmkvsads sdldyfntls rqhlalllle pddqkrinlv ntfshyitga
481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk
541 amvsawiysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk
601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr
661 ygryqshgva qivsngsqls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
781 vettlfqhai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq
841 hqvsaenknr qptegnfssa widhstrpkd asyeymvfld atpekmgema qkfrenngly
901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk pa

SEQ ID NO: 5 Chondroitinase AC protein Locus 1HMW_A

ORIGIN
1 mkklfvtciv ffsilspall iaqqtgtael imkrvmldlk kplrnmdkva eknlntlqpd
61 gswkdvpykd damtnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
121 pksrnwwhne iatpqalgem lilmrygkkp ldealvhklt ermkrgepek ktganktdia
181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
301 ekkrllvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
361 rmvskrtrrs esgnkenllg rylsdgatni qlrgpeyyni mpvwewdkip gitsrdyltd
421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna
481 penitttlnq swlngpvist agktgrgkit tfkaqgqfwl lhdaigyyfp eganlslstq

541 sqkgnwfhin nshskdevsg dvfklwinhg arpenaqyay ivlpginkpe eikkyngtap
601 kvlantnqlq avyhqqldmv qaifytagkl svagieietd kpcavlikhi ngkqviwaad
661 plqkektavl sirdlktgkt nrvkidfpqq efagatvelk

SEQ ID NO: 6 CΔ200 AC (Q_{23}-T_{500}) protein

qqtgtael imkrvmldlk kplrnmdkva eknlntlqpd
61 gswkdvpykd damtnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
121 pksrnwwhne iatpqalgem lilmrygkkp ldealvhklt ermkrgepek ktganktdia
181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
301 ekkrllvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
361 rmvskrtrrs esgnkenllg rylsdgatni qlrgpeyyni mpvwewdkip gitsrdyltd
421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna
481 penitttlnq swlngpvist

SEQ ID NO: 7 C**Δ220** AC (Q_{23}-A_{480}) protein

qqtgtael imkrvmldlk kplrnmdkva eknlntlqpd
61 gswkdvpykd damtnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
121 pksrnwwhne iatpqalgem lilmrygkkp ldealvhklt ermkrgepek ktganktdia
181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
301 ekkrllvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
361 rmvskrtrrs esgnkenllg rylsdgatni qlrgpeyyni mpvwewdkip gitsrdyltd
421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna

SEQ ID NO: 8 NΔ20 CΔ200 AC (L_{43}-T_{500}) protein

lrnmdkva eknlntlqpd
61 gswkdvpykd damtnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
121 pksrnwwhne iatpqalgem lilmrygkkp ldealvhklt ermkrgepek ktganktdia
181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
301 ekkrllvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
361 rmvskrtrrs esgnkenllg rylsdgatni qlrgpeyyni mpvwewdkip gitsrdyltd
421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna
481 penitttlnq swlngpvist

SEQ ID NO: 9 NΔ50 CΔ200 AC (T_{74}-T_{500}) protein

tnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
121 pksrnwwhne iatpqalgem lilmrygkkp ldealvhklt ermkrgepek ktganktdia
181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
301 ekkrllvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
361 rmvskrtrrs esgnkenllg rylsdgatni qlrgpeyyni mpvwewdkip gitsrdyltd
421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna
481 penitttlnq swlngpvist

SEQ ID NO: 10 NΔ100 CΔ200 AC (S_{123}-T_{500}) protein

srnwwhne iatpqalgem lilmrygkkp ldealvhklt ermkrgepek ktganktdia
181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
301 ekkrllvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
361 rmvskrtrrs esgnkenllg rylsdgatni qlrgpeyyni mpvwewdkip gitsrdyltd
421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna
481 penitttlnq swlngpvist

SEQ ID NO: 11 NΔ50 CΔ275 AC (T_{74}-L_{426}) protein

tnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
121 pksrnwwhne iatpqalgem lilmrygkkp ldealvhklt ermkrgepek ktganktdia
181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
301 ekkrllvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
361 rmvskrtrrs esgnkenllg rylsdgatni qlrgpeyyni mpvwewdkip gitsrdyltd
421 rpltkl

SEQ ID NO: 12 Chondroitinase B Locus Q46079 protein

ORIGIN
1 mkmlnklagy llpimvllnv apclgqvvas netlyqvvke vkpgglvqia dgtykdvqli
61 vsnsgksglp itikalnpgk vfftgdakve lrgehlileg iwfkdgnrai qawkshgpgl
121 vaiygsynri tacvfdcfde ansayittsl tedgkvpqhc ridhcsftdk itfdqvinln
181 ntaraikdgs vggpgmyhrv dhcffsnpqk pgnagggiri gyyrndigrc lvdsnlfmrq
241 dseaeiitsk sqenvyygnt ylncqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
301 rhviacnyfe lsetiksrgn aalylnpgam asehalafdm liannafinv ngyaihfnpl
361 derrkeycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve
421 kgipvnisan rsaykpvkik diqpiegial dlnaliskgi tgkplswdev rpywlkempg
481 tyaltarlsa draakfkavi krnkeh

SEQ ID NO: 13 NΔ80 Chase B (G_{106}-H_{506}) protein

gnrai qawkshgpgl
121 vaiygsynri tacvfdcfde ansayittsl tedgkvpqhc ridhcsftdk itfdqvinln
181 ntaraikdgs vggpgmyhrv dhcffsnpqk pgnagggiri gyyrndigrc lvdsnlfmrq
241 dseaeiitsk sqenvyygnt ylncqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
301 rhviacnyfe lsetiksrgn aalylnpgam asehalafdm liannafinv ngyaihfnpl
361 derrkeycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve
421 kgipvnisan rsaykpvkik diqpiegial dlnaliskgi tgkplswdev rpywlkempg
481 tyaltarlsa draakfkavi krnkeh

SEQ ID NO: 14 NΔ120 Chase B (I_{146}-H_{506}) protein

ittsl tedgkvpqhc ridhcsftdk itfdqvinln
181 ntaraikdgs vggpgmyhrv dhcffsnpqk pgnagggiri gyyrndigrc lvdsnlfmrq
241 dseaeiitsk sqenvyygnt ylncqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
301 rhviacnyfe lsetiksrgn aalylnpgam asehalafdm liannafinv ngyaihfnpl

361 derrkeycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve
421 kgipvnisan rsaykpvkik diqpiegial dlnaliskgi tgkplswdev rpywlkempg
481 tyaltarlsa draakfkavi krnkeh

SEQ ID NO: 15 CΔ19 Chase B (Q_{26}-L_{488}) protein

qvvas netlyqvvke vkpgglvqia dgtykdvqli
61 vsnsgksglp itikalnpgk vfftgdakve lrgehlileg iwfkdgnrai qawkshgpgl
121 vaiygsynri tacvfdcfde ansayittsl tedgkvpqhc ridhcsftdk itfdqvinln
181 ntaraikdgs vggpgmyhrv dhcffsnpqk pgnagggiri gyyrndigrc lvdsnlfmrq
241 dseaeiitsk sqenvyygnt ylncqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
301 rhviacnyfe lsetiksrgn aalylnpgam asehalafdm liannafinv ngyaihfnpl
361 derrkeycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve
421 kgipvnisan rsaykpvkik diqpiegial dlnaliskgi tgkplswdev rpywlkempg
481 tyaltarl

SEQ ID NO: 16 CΔ120 Chase B (Q_{26}-K_{390}) protein

qvvas netlyqvvke vkpgglvqia dgtykdvqli
61 vsnsgksglp itikalnpgk vfftgdakve lrgehlileg iwfkdgnrai qawkshgpgl
121 vaiygsynri tacvfdcfde ansayittsl tedgkvpqhc ridhcsftdk itfdqvinln
181 ntaraikdgs vggpgmyhrv dhcffsnpqk pgnagggiri gyyrndigrc lvdsnlfmrq
241 dseaeiitsk sqenvyygnt ylncqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
301 rhviacnyfe lsetiksrgn aalylnpgam asehalafdm liannafinv ngyaihfnpl
361 derrkeycaa nrlkfetphq lmlkgnlffk

SEQ ID NO: 17 NΔ120 CΔ120 Chase B (I_{146}-K_{390}) protein

ittsl tedgkvpqhc ridhcsftdk itfdqvinln
181 ntaraikdgs vggpgmyhrv dhcffsnpqk pgnagggiri gyyrndigrc lvdsnlfmrq
241 dseaeiitsk sqenvyygnt ylncqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
301 rhviacnyfe lsetiksrgn aalylnpgam asehalafdm liannafinv ngyaihfnpl
361 derrkeycaa nrlkfetphq lmlkgnlffk

SEQ ID NO: 18 Choindroitinase AC nucleotide locus CHU27583

ORIGIN
1 atgaagaaat tatttgtaac ctgtatagtc tttttctcta ttttaagtcc tgctctgctt
61 attgcacagc agaccggtac tgcagaactg attatgaagc gggtgatgct ggaccttaaa
121 aagcctttgc gcaatatgga taaggtggcg gaaaagaacc tgaatacgct gcagcctgac
181 ggtagctgga aggatgtgcc ttataaagat gatgccatga ccaattggtt gccaaacaac
241 cacctgctac aattggaaac tattatacag gcttatattg aaaaagatag tcactattat
301 ggcgacgata aagtgtttga ccagatttcc aaagctttta agtattggta tgacagcgac
361 ccgaaaagcc gcaactggtg gcacaatgaa attgccactc cgcaggccct tggtgaaatg
421 ctgatcctga tgcgttacgg taaaaagccg cttgatgaag cattggtgca taaattgacc
481 gaaagaatga agcggggcga accggagaag aaaacggggg ccaacaaaac agatatcgcc
541 ctgcattact tttatcgtgc tttgttaacg tctgatgagg ctttgctttc cttcgccgta
601 aaagaattgt tttatcccgt acagtttgta cactatgagg aaggcctgca atacgattat
661 tcctacctgc agcacggtcc gcaattacag atatcgagct acggtgccgt atttattacc
721 ggggtactga aacttgccaa ttacgttagg gataccccct atgctttaag taccgagaaa
781 ctggctatat tttcaaagta ttaccgcgac agttatctga aagctatccg tggaagttat

841 atggatttta acgtagaagg ccgcggagta agccggccag acattctaaa taaaaaggca
901 gaaaaaaaga ggttgctggt ggcgaagatg atcgatctta agcatactga agaatgggct
961 gatgcgatag ccaggacaga tagcacagtt gcggccggct ataagattga gccctatcac
1021 catcagttct ggaatggtga ttatgtgcaa catttaagac ctgcctattc ttttaatgtt
1081 cgtatggtga gtaagcggac ccgacgcagt gaatccggca ataaagaaaa cctgctgggc
1141 aggtatttat ctgatggggc tactaacata caattgcgcg gaccagaata ctataacatt
1201 atgccggtat gggaatggga caagattcct ggcataacca gccgtgatta tttaaccgac
1261 agacctttga cgaagctttg gggagagcag gggagcaatg actttgcagg aggggtgtct
1321 gatggtgtat acggggccag tgcctacgca ttggattacg atagcttaca ggcaaagaaa
1381 gcctggttct tttttgacaa agagattgta tgtcttggtg ccggtatcaa cagcaatgcc
1441 cctgaaaaca ttaccactac ccttaaccag agctggttaa atggcccggt tataagtact
1501 gcaggtaaaa ccggccgggg taaaataaca acgtttaaag cacagggaca gttctggttg
1561 ttgcacgatg cgattggtta ttactttcct gaaggggcca accttagtct gagtacccag
1621 tcgcaaaaag gcaattggtt ccacatcaac aattcacatt caaaagatga agtttctggt
1681 gatgtattta agctttggat caaccatggt gccaggccag aaaatgcgca gtatgcttat
1741 atcgttttgc cgggaataaa caagccggaa gaaattaaaa aatataatgg aacggcaccg
1801 aaagtccttg ccaataccaa ccagctgcag gcagtttatc atcagcagtt agatatggta
1861 caggctatct tctatacagc tggaaaatta agcgtagcgg gcatagaaat tgaaacagat
1921 aagccatgtg cagtgctgat caagcacatc aatggcaagc aggtaatttg ggctgccgat
1981 ccattgcaaa aagaaaagac tgcagtgttg agcatcaggg atttaaaaac aggaaaaaca
2041 aatcgggtaa aaattgattt tccgcaacag gaatttgcag gtgcaacggt tgaactgaaa
2101 tag
//

SEQ ID NO: 19 Chondriotinase AC nucleic acid deletion NΔ50 C Δ275 ($a_{220} - t_{1278}$)

atgccatga ccaattggtt gccaaacaac
241 cacctgctac aattggaaac tattatacag gcttatattg aaaaagatag tcactattat
301 ggcgacgata aagtgtttga ccagatttcc aaagctttta agtattggta tgacagcgac
361 ccgaaaagcc gcaactggtg gcacaatgaa attgccactc cgcaggccct tggtgaaatg
421 ctgatcctga tgcgttacgg taaaaagccg cttgatgaag cattggtgca taaattgacc
481 gaaagaatga agcggggcga accggagaag aaaacggggg ccaacaaaac agatatcgcc
541 ctgcattact tttatcgtgc tttgttaacg tctgatgagg ctttgctttc cttcgccgta
601 aaagaattgt tttatcccgt acagtttgta cactatgagg aaggcctgca atacgattat
661 tcctacctgc agcacggtcc gcaattacag atatcgagct acggtgccgt atttattacc
721 ggggtactga aacttgccaa ttacgttagg gataccccct atgctttaag taccgagaaa
781 ctggctatat tttcaaagta ttaccgcgac agttatctga aagctatccg tggaagttat
841 atggatttta acgtagaagg ccgcggagta agccggccag acattctaaa taaaaaggca
901 gaaaaaaaga ggttgctggt ggcgaagatg atcgatctta agcatactga agaatgggct
961 gatgcgatag ccaggacaga tagcacagtt gcggccggct ataagattga gccctatcac
1021 catcagttct ggaatggtga ttatgtgcaa catttaagac ctgcctattc ttttaatgtt
1081 cgtatggtga gtaagcggac ccgacgcagt gaatccggca ataaagaaaa cctgctgggc
1141 aggtatttat ctgatggggc tactaacata caattgcgcg gaccagaata ctataacatt
1201 atgccggtat gggaatggga caagattcct ggcataacca gccgtgatta tttaaccgac
1261 agacctttga cgaagctt

SEQ ID NO: 20 Chondroitinase B nuclei acid Locus CHU27584

ORIGIN
1 atgaagatgc tgaataaact agccggatac ttattgccga tcatggtgct gctgaatgtg
61 gcaccatgct taggtcaggt tgttgcttca aatgaaactt tataccaggt tgtaaaggag

121 gtaaaacccg gtggtctggt acagattgcc gatgggactt ataaagatgt tcagctgatt
181 gtcagcaatt caggaaaatc tggtttgccc atcactatta aagccctgaa cccgggtaag
241 gttttttta ccggagatgc taaagtagag ctgaggggcg agcacctgat actggaaggc
301 atctggttta aagacgggaa cagagctatt caggcatgga aatcacatgg acccggattg
361 gtggctatat atggtagcta taaccgcatt accgcatgtg tatttgattg ttttgatgaa
421 gccaattctg cttacattac tacttcgctt accgaagacg gaaaggtacc tcaacattgc
481 cgcatagacc attgcagttt taccgataag atcacttttg accaggtaat taacctgaac
541 aatacagcca gagctattaa agacggttcg gtgggaggac cggggatgta ccatcgtgtt
601 gatcactgtt ttttttccaa tccgcaaaaa ccgggtaatg ccggaggggg aatcaggatt
661 ggctattacc gtaatgatat aggccgttgt ctggtagact ctaacctgtt tatgcgtcag
721 gattcggaag cagagatcat caccagcaaa tcgcaggaaa atgtttatta tggtaatact
781 tacctgaatt gccagggcac catgaacttt cgtcacggtg atcatcaggt ggccattaac
841 aatttttata taggcaatga ccagcgattt ggatacgggg gaatgtttgt ttggggaagc
901 aggcatgtca tagcctgtaa ttattttgag ctgtccgaaa ccataaagtc gaggggggaac
961 gccgcattgt atttaaaccc cggtgctatg gcttcggagc atgctcttgc tttcgatatg
1021 ttgatagcca acaacgcttt catcaatgta aatgggtatg ccatccattt taatccattg
1081 gatgagcgca gaaaagaata ttgtgcagcc aataggctta agttcgaaac cccgcaccag
1141 ctaatgttaa aaggcaatct tttctttaag gataaacctt atgtttaccc atttttaaa
1201 gatgattatt ttatagcagg gaaaaatagc tggactggta atgtagcctt aggtgtggaa
1261 aagggaatcc ctgttaacat ttcggccaat aggtctgcct ataagccggt aaaaattaaa
1321 gatatccagc ccatagaagg aatcgctctt gatctcaatg cgctgatcag caaaggcatt
1381 acaggaaagc cccttagctg ggatgaagta aggccctact ggttaaaaga aatgcccggg
1441 acgtatgctt taacggccag gctttctgca gatagggctg caaagtttaa agccgtaatt
1501 aaaagaaata aagagcactg a

SEQ ID NO: 21 Chondriotinase B nucleic acid deletion NΔ120 CΔ120 ($a_{436} - g_{1170}$)

attac tacttcgctt accgaagacg gaaaggtacc tcaacattgc
481 cgcatagacc attgcagttt taccgataag atcacttttg accaggtaat taacctgaac
541 aatacagcca gagctattaa agacggttcg gtgggaggac cggggatgta ccatcgtgtt
601 gatcactgtt ttttttccaa tccgcaaaaa ccgggtaatg ccggaggggg aatcaggatt
661 ggctattacc gtaatgatat aggccgttgt ctggtagact ctaacctgtt tatgcgtcag
721 gattcggaag cagagatcat caccagcaaa tcgcaggaaa atgtttatta tggtaatact
781 tacctgaatt gccagggcac catgaacttt cgtcacggtg atcatcaggt ggccattaac
841 aatttttata taggcaatga ccagcgattt ggatacgggg gaatgtttgt ttggggaagc
901 aggcatgtca tagcctgtaa ttattttgag ctgtccgaaa ccataaagtc gaggggggaac
961 gccgcattgt atttaaaccc cggtgctatg gcttcggagc atgctcttgc tttcgatatg
1021 ttgatagcca acaacgcttt catcaatgta aatgggtatg ccatccattt taatccattg
1081 gatgagcgca gaaaagaata ttgtgcagcc aataggctta agttcgaaac cccgcaccag
1141 ctaatgttaa aaggcaatct tttctttaag

SEQ ID NO: 22 Chondroitinase ABCI nucleic acid Locus I29953

ORIGIN
1 ggaattccat cactcaatca ttaaatttag gcacaacgat gggctatcag cgttatgaca
61 aatttaatga aggacgcatt ggtttcactg ttagccagcg tttctaagga gaaaaataat
121 gccgatattt cgttttactg cacttgcaat gacattgggg ctattatcag cgccttataa
181 cgcgatggca gccaccagca atcctgcatt tgatcctaaa aatctgatgc agtcagaaat
241 ttaccatttt gcacaaaata acccattagc agacttctca tcagataaaa actcaatact
301 aacgttatct gataaacgta gcattatggg aaaccaatct cttttatgga aatggaaagg

361 tggtagtagc tttactttac ataaaaaact gattgtcccc accgataaag aagcatctaa
421 agcatgggga cgctcatcta cccccgtttt ctcattttgg ctttacaatg aaaaaccgat
481 tgatggttat cttactatcg atttcggaga aaaactcatt tcaaccagtg aggctcaggc
541 aggctttaaa gtaaaattag atttcactgg ctggcgtgct gtgggagtct ctttaaataa
601 cgatcttgaa aatcgagaga tgaccttaaa tgcaaccaat acctcctctg atggtactca
661 agacagcatt gggcgttctt taggtgctaa agtcgatagt attcgtttta aagcgccttc
721 taatgtgagt cagggtgaaa tctatatcga ccgtattatg ttttctgtcg atgatgctcg
781 ctaccaatgg tctgattatc aagtaaaaac tcgcttatca gaacctgaaa ttcaatttca
841 caacgtaaag ccacaactac ctgtaacacc tgaaaattta gcggccattg atcttattcg
901 ccaacgtcta attaatgaat ttgtcggagg tgaaaaagag acaaacctcg cattagaaga
961 gaatatcagc aaattaaaaa gtgatttcga tgctcttaat attcacactt tagcaaatgg
1021 tggaacgcaa ggcagacatc tgatcactga taaacaaatc attatttatc aaccagagaa
1081 tcttaactcc caagataaac aactatttga taattatgtt attttaggta attacacgac
1141 attaatgttt aatattagcc gtgcttatgt gctggaaaaa gatcccacac aaaaggcgca
1201 actaaagcag atgtacttat taatgacaaa gcatttatta gatcaaggct ttgttaaagg
1261 gagtgcttta gtgacaaccc atcactgggg atacagttct cgttggtggt atatttccac
1321 gttattaatg tctgatgcac taaaagaagc gaacctacaa actcaagttt atgattcatt
1381 actgtggtat tcacgtgagt ttaaaagtag ttttgatatg aaagtaagtg ctgatagctc
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1561 aacgcaagtg ccaccgggtg gtaaagatgg tttacgccct gatggtacag catggcgaca
1621 tgaaggcaac tatccgggct actctttccc agcctttaaa aatgcctctc agcttattta
1681 tttattacgc gatacaccat tttcagtggg tgaaagtggt tggaataacc tgaaaaaagc
1741 gatggtttca gcgtggatct acagtaatcc agaagttgga ttaccgcttg caggaagaca
1801 ccctttaac tcaccttcgt taaaatcagt cgctcaaggc tattactggc ttgccatgtc
1861 tgcaaaatca tcgcctgata aaacacttgc atctatttat cttgcgatta gtgataaaac
1921 acaaaatgaa tcaactgcta tttttggaga aactattaca ccagcgtctt tacctcaagg
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2041 actgaaagct tataacacca atgtttggtc atctgaaatt tataacaaag ataaccgtta
2101 tggccgttac caaagtcatg gtgtcgctca aatagtgagt aatggctcgc agctttcaca
2161 gggctatcag caagaaggtt gggattggaa tagaatgcaa ggggcaacca ctattcacct
2221 tcctcttaaa gacttagaca gtcctaaacc tcatacctta atgcaacgtg gagagcgtgg
2281 atttagcgga acatcatccc ttgaaggtca atatggcatg atggcattcg atcttattta
2341 tcccgccaat cttgagcgtt ttgatcctaa tttcactgcg aaaaagagtg tattagccgc
2401 tgataatcac ttaattttta ttggtagcaa tataaatagt agtgataaaa ataaaaatgt
2461 tgaaacgacc ttattccaac atgccattac tccaacatta aatacccttt ggattaatgg
2521 acaaaagata gaaaacatgc cttatcaaac aacacttcaa caaggtgatt ggttaattga
2581 tagcaatggc aatggttact taattactca agcagaaaaa gtaaatgtaa gtcgccaaca
2641 tcaggtttca gcggaaaata aaaatcgcca accgacagaa ggaaacttta gctcggcatg
2701 gatcgatcac agcactcgcc ccaaagatgc cagttatgag tatatggtct ttttagatgc
2761 gacacctgaa aaaatgggag agatggcaca aaaattccgt gaaaataatg ggttatatca
2821 ggttcttcgt aaggataaag acgttcatat tattctcgat aaactcagca atgtaacggg
2881 atatgccttt tatcagccag catcaattga agacaaatgg atcaaaaagg ttaataaacc
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3121 tgaactgacg tttacgagtt actttggtat tccacaagaa atcaaactct cgccactccc
3181 ttgatttaat caaaagaacg ctcttgcgtt ccttttttat ttgcaggaaa tctgattatg
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3301 caagcattac ccactctgtc tcatgaagct ttcggcgata tttatctttt tgaaggtgaa

3361 ttacccaata cccttaccac ttcaaataat aatcaattat cgctaagcaa acagcatgct
3421 aaagatggtg aacaatcact caaatggcaa tatcaaccac aagcaacatt aacactaaat
3481 aatattgtta attaccaaga tgataaaaat acagccacac cactcacttt tatgatgtgg
3541 atttataatg aaaaacctca atcttcccca ttaacgttag catttaaaca aaataataaa
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3661 tttcgtgata tgcaaggctc tgcgacaggt caacttgatc aattagtgat caccgctcca
3721 aaccaagccg gaacactctt ttttgatcaa atcatcatga gtgtaccgtt agacaatcgt
3781 tgggcagtac ctgactatca aacaccttac gtaaataacg cagtaaacac gatggttagt
3841 aaaaactgga gtgcattatt gatgtacgat cagatgtttc aagcccatta ccctacttta
3901 aacttcgata ctgaatttcg cgatgaccaa acagaaatgg cttcgattta tcagcgcttt
3961 gaatattatc aaggaattcc
//

SEQ ID NO: 23 TAT fusion chondroitinase ABCI nucleic acid

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tggtggtggtggtggtgccaccagca atcctgcatt tgatcctaaa aatctgatgc agtcagaaat
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301 aacgttatct gataaacgta gcattatggg aaaccaatct cttttatgga aatggaaagg
361 tggtagtagc tttactttac ataaaaaact gattgtcccc accgataaag aagcatctaa
421 agcatgggga cgctcatcta cccccgtttt ctcattttgg ctttacaatg aaaaaccgat
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541 aggctttaaa gtaaaattag atttcactgg ctggcgtgct gtgggagtct ctttaaataa
601 cgatcttgaa aatcgagaga tgaccttaaa tgcaaccaat acctcctctg atggtactca
661 agacagcatt gggcgttctt taggtgctaa agtcgatagt attcgtttta aagcgccttc
721 taatgtgagt cagggtgaaa tctatatcga ccgtattatg ttttctgtcg atgatgctcg
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901 ccaacgtcta attaatgaat ttgtcggagg tgaaaaagag acaaacctcg cattagaaga
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2101 tggccgttac caaagtcatg gtgtcgctca aatagtgagt aatggctcgc agctttcaca
2161 gggctatcag caagaaggtt gggattggaa tagaatgcaa ggggcaacca ctattcacct
2221 tcctcttaaa gacttagaca gtcctaaacc tcataccta atgcaacgtg gagagcgtgg

2281 atttagcgga acatcatccc ttgaaggtca atatggcatg atggcattcg atcttattta
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2401 tgataatcac ttaattttta ttggtagcaa tataaatagt agtgataaaa ataaaaatgt
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2521 acaaaagata gaaaacatgc cttatcaaac aacacttcaa caaggtgatt ggttaattga
2581 tagcaatggc aatggttact taattactca agcagaaaaa gtaaatgtaa gtcgccaaca
2641 tcaggtttca gcggaaaata aaaatcgcca accgacagaa ggaaacttta gctcggcatg
2701 gatcgatcac agcactcgcc ccaaagatgc cagttatgag tatatggtct ttttagatgc
2761 gacacctgaa aaaatgggag agatggcaca aaaattccgt gaaaataatg ggttatatca
2821 ggttcttcgt aaggataaag acgttcatat tattctcgat aaactcagca atgtaacggg
2881 atatgccttt tatcagccag catcaattga agacaaatgg atcaaaaagg ttaataaacc
2941 tgcaattgtg atgactcatc gacaaaaaga cactcttatt gtcagtgcag ttacacctga
3001 tttaaatatg actcgccaaa aagcagcaac tcctgtcacc atcaatgtca cgattaatgg
3061 caaatggcaa tctgctgata aaaatagtga agtgaaatat caggtttctg gtgataacac
3121 tgaactgacg tttacgagtt actttggtat tccacaagaa atcaaactct cgccactccc
3181 ttgatttaat caaaagaacg ctcttgcgtt ccttttttat ttgcaggaaa tctgattatg
3241 ctaataaaaa acccctttagc ccacgcggtt acattaagcc tctgtttatc attacccgca
3301 caagcattac ccactctgtc tcatgaagct ttcggcgata tttatctttt tgaaggtgaa
3361 ttacccaata cccttaccac ttcaaataat aatcaattat cgctaagcaa acagcatgct
3421 aaagatggtg aacaatcact caaatggcaa tatcaaccac aagcaacatt aacactaaat
3481 aatattgtta attaccaaga tgataaaaat acagccacac cactcacttt tatgatgtgg
3541 atttataatg aaaaacctca atcttcccca ttaacgttag catttaaaca aaataataaa
3601 attgcactaa gttttaatgc tgaacttaat tttacggggt ggcgaggtat tgctgttcct
3661 tttcgtgata tgcaaggctc tgcgacaggt caacttgatc aattagtgat caccgctcca
3721 aaccaagccg gaacactctt ttttgatcaa atcatcatga gtgtaccgtt agacaatcgt
3781 tgggcagtac ctgactatca aacaccttac gtaaataacg cagtaaacac gatggttagt
3841 aaaaactgga gtgcattatt gatgtacgat cagatgtttc aagcccatta ccctacttta
3901 aacttcgata ctgaatttcg cgatgaccaa acagaaatgg cttcgattta tcagcgcttt
3961 gaatattatc aaggaattcc

SEQ ID NO: 24 An HIV TAT sequence and Gly penta linker protein

G R K K R R Q R R R G G G G G

SEQ ID NO: 25 Tat sequence and Gly penta linker nucleic acid

ggt cgt aaa aag cgt cgt caa cgt cgt cgt ggt ggt ggt ggt ggt

SEQ ID NO: 26 Present Invention Chondroitinase ABC II Nucleic acid

ttacccactctgtctcatgaagctttcggcgatatttatctttttgaaggcgaattacccaatatccttaccacttcaaataataatcaattatcg
ctaagcaaacagcatgctaaagatggtgaacaatcactcaaatggcaatatcaaccacaagcaacattaacactaaataatattgttaatt
accaagatgataaaaatacagccacaccactcacttttatgatgtggatttataatgaaaaacctcaatcttccccattaacgttagcattta
aacaaaataataaaattgcactaagttttaatgctgaacttaattttacggggtggcgaggtattgctgttcctttcgtgatatgcaaggctc
tgcgacaggtcaacttgatcaattagtgatcaccgctccaaaccaagccggaacactctttttgatcaaatcatcatgagtgtaccgtta
gacaatcgttgggcagtacctgactatcaaacaccttacgtaaataacgcagtaaacacgatggttagtaaaaactggagtgcattattg
atgtacgatcagatgtttcaagcccattaccctactttaaacttcgatactgaatttcgcgatgaccaaacagaaatggcttcgatttatcag
cgctttgaatattatcaaggaattcgtagtgataaaaaaattactccagatatgctagataaacatttagcgttatgggaaaaattggggtta
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ggggactcaaaaagcattacttgatgccaatatgctaagagatgtgggcaaaacgcttcttcaaactgctatttacttgcgtagcgattca
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tcaaattattactcatgttggttaccaaaccagagaacttttgatgcatggtttattggccgtcatgttcttgcaaaaaataacctttagccc
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actcaattgcaatggatgataaaaagcttattgatgctaccggattatcaacaacgtcaacaagccttagcgcaactgcaaagttggcta
aataaaaccattctaagctcaaaaggtgttgctggcggtttcaaatctgatggttctatttttcaccattcacaacattaccccgcttatgcta
aagatgcatttggtggtttagcacccagtgtttatgcattaagtgattcacctttcgcttatctacttcagcacatgagcatttaaaagatgtt
ttgttaaaaatgcggatctacaccaaagagacacaaattcctgtggtattaagtggtcgtcatccaactgggttgcataaaatagggatcg
cgccatttaaatggatggcattagcaggaaccccagatggcaaacaaaagttagataccacattatccgccgcttatgcaaacttagac
aacaaaacgcattttgaaggcattaacgctgaaagtgagccagtcggcgcatgggcaatgaattatgcatcaatggcaatacaacgaa
gagcatcgacccaatcaccacaacaaagctggctcgccatagcgcgcggttttagccgttatcttgttggtaatgaaagctatgaaaata
acaaccgttatggtcgttatttacaatatggacaattggaaattattccagctgatttaactcaatcagggtttagccatgctggatgggatt
ggaatagatatccaggtacaacaactattcatcttccctataacgaacttgaagcaaaacttaatcaattacctgctgcaggtattgaaga
aatgttgctttcaacagaaagttactctggtgcaaataccccttaataataacagtatgtttgccatgaaattacacggtcacagtaaatatca
acaacaaagcttaagggcaaataaatcctatttcttatttgataatagagttattgctttaggctcaggtattgaaaatgatgataaacaacat
acgaccgaaacaacactattccagtttgccgtccctaaattacagtcagtgatcattaatggcaaaaaggtaaatcaattagatactcaatt
aactttaaataatgcagatacattaattgatcctgccggcaatttatataagctcactaaaggacaaactgtaaaatttagttatcaaaaaca
acattcacttgatgatagaaattcaaaaccaacagaacaattatttgcaacagctgttatttctcatggtaaggcaccgagtaatgaaaatt
atgaatatgcaatagctatcgaagcacaaaataataaagctcccaaatacacagtattacaacataatgatcagctccatgcggtaaaag
ataaaataacccaagaagagggatatggtttttttgaagccactaagttaaaatcagcggatgcaacattattatccagtgatgcgccggt
tatggtcatggctaaaatacaaaatcagcaattaacattaagtattgttaatcctgatttaaatttatatcaaggtagagaaaaagatcaattt
gatgataaaggtaatcaaatcgaagttagtgtttattctcgtcattggcttacagcagaatcgcaatcaacaaatagtactattaccgtaaa
aggaatatggaaattaacgacacctcaacccggtgttattattaagcaccacaataacaacactcttattacgacaacaaccatacaggc
aacacctactgttattaatttagttaagtaa

SEQ ID NO: 27 Present Invention Chondroitinase ABC II protein

```
>_ ABC (present invention)                                          990 aa vs.
>_ ABC (mature)                                                     990 aa
scoring matrix: , gap penalties: -12/-2
98.3% identity;        Global alignment score: 6393

               10        20        30        40        50        60
457676 LPTLSHEAFGDIYLFEGELPNILTTSNNNQLSLSKQHAKDGEQSLKWQYQPQATLTLNNI
       :::::::::::::::::::: :::::::::::::::::::::::::::::::::::::
_      LPTLSHEAFGDIYLFEGELPNTLTTSNNNQLSLSKQHAKDGEQSLKWQYQPQATLTLNNI
               10        20        30        40        50        60

               70        80        90       100       110       120
457676 VNYQDDKNTATPLTFMMWIYNEKPQSSPLTLAFKQNNKIALSFNAELNFTGWRGIAVPFR
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      VNYQDDKNTATPLTFMMWIYNEKPQSSPLTLAFKQNNKIALSFNAELNFTGWRGIAVPFR
               70        80        90       100       110       120

              130       140       150       160       170       180
457676 DMQGSATGQLDQLVITAPNQAGTLFFDQIIMSVPLDNRWAVPDYQTPYVNNAVNTMVSKN
       :::::.::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      DMQGSVTGQLDQLVITAPNQAGTLFFDQIIMSVPLDNRWAVPDYQTPYVNNAVNTMVSKN
              130       140       150       160       170       180

              190       200       210       220       230       240
457676 WSALLMYDQMFQAHYPTLNFDTEFRDDQTEMASIYQRFEYYQGIRSDKKITPDMLDKHLA
       :::::::::::::::::::::::::::::::: :::::::::::::::::::::::::::
```

```
_       WSALLMYDQMFQAHYPTLNFDTEFRDDQTEMASRYQRFEYYQGIRSDKKITPDMLDKHLA
               190       200       210       220       230       240

               250       260       270       280       290       300
457676 LWEKLGLTQHADGSITGKALDHPNRQHFMKVEGVFSEGTQKALLDANMLRDVGKTLLQTA
       ::::: :::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      LWEKLVLTQHADGSITGKALDHPNRQHFMKVEGVFSEGTQKALLDANMLRDVGKTLLQTA
               250       260       270       280       290       300

               310       320       330       340       350       360
457676 IYLRSDSLSATGRKKLEERYLLGTRYVLEQGFTRGSGYQIITHVGYQTRELFDAWFIGRH
       :::::::::: :::::::::::::::::::: ::::::::.::::::::::::::::::::
_      IYLRSDSLSATDRKKLEERYLLGTRYVLEQGFHRGSGYQIISHVGYQTRELFDAWFIGRH
               310       320       330       340       350       360

               370       380       390       400       410       420
457676 VLAKNNLLAPTQQAMMWYNATGRIFEKDNEIVDANVDILNTQLQWMIKSLLMLPDYQQRQ
       ::::::::::::::::::::::::::.::::::::::::::::::::::::::::::::::
_      VLAKNNLLAPTQQAMMWYNATGRIFEKNNEIVDANVDILNTQLQWMIKSLLMLPDYQQRQ
               370       380       390       400       410       420

               430       440       450       460       470       480
457676 QALAQLQSWLNKTILSSKGVAGGFKSDGSIFHHSQHYPAYAKDAFGGLAPSVYALSDSPF
       ::::::: :::::::::::::::::::::::::::::::::::::::::::::::::::::
_      QALAQLQRWLNKTILSSKGVAGGFKSDGSIFHHSQHYPAYAKDAFGGLAPSVYALSDSPF
               430       440       450       460       470       480

               490       500       510       520       530       540
457676 RLSTSAHEHLKDVLLKMRIYTKETQIPVVLSGRHPTGLHKIGIAPFKWMALAGTPDGKQK
       ::::::::.::::::::::::::::::.::::::::::::::::::::::::::::::::::
_      RLSTSAHERLKDVLLKMRIYTKETQIPAVLSGRHPTGLHKIGIAPFKWMALAGTPDGKQK
               490       500       510       520       530       540

               550       560       570       580       590       600
457676 LDTTLSAAYANLDNKTHFEGINAESEPVGAWAMNYASMAIQRRASTQSPQQSWLAIARGF
       ::::::::::.::::::::::.::::::::::::::::::::::::::::::::::::::::
_      LDTTLSAAYAKLDNKTHFEGIKAESEPVGAWAMNYASMAIQRRASTQSPQQSWLAIARGF
               550       560       570       580       590       600

               610       620       630       640       650       660
457676 SRYLVGNESYENNNRYGRYLQYGQLEIIPADLTQSGFSHAGWDWNRYPGTTTIHLPYNEL
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      SRYLVGNESYENNNRYGRYLQYGQLEIIPADLTQSGFSHAGWDWNRYPGTTTIHLPYNEL
               610       620       630       640       650       660

               670       680       690       700       710       720
457676 EAKLNQLPAAGIEEMLLSTESYSGANTLNNNSMFAMKLHGHSKYQQQSLRANKSYFLFDN
       :::::::::::::::::::::::::::::::::::::::: :::::::::::::::::::
_      EAKLNQLPAAGIEEMLLSTESYSGANTLNNNSMFAMKLHGPSKYQQQSLRANKSYFLFDN
               670       680       690       700       710       720

               730       740       750       760       770       780
457676 RVIALGSGIENDDKQHTTETTLFQFAVPKLQSVIINGKKVNQLDTQLTLNNADTLIDPAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      RVIALGSGIENDDKQHTTETTLFQFAVPKLQSVIINGKKVNQLDTQLTLNNADTLIDPAG
               730       740       750       760       770       780

               790       800       810       820       830       840
457676 NLYKLTKGQTVKFSYQKQHSLDDRNSKPTEQLFATAVISHGKAPSNENYEYAIAIEAQNN
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      NLYKLTKGQTVKFSYQKQHSLDDRNSKPTEQLFATAVISHGKAPSNENYEYAIAIEAQNN
```

```
              790        800        810        820        830        840

              850        860        870        880        890        900
457676 KAPKYTVLQHNDQLHAVKDKITQEEGYGFFEATKLKSADATLLSSDAPVMVMAKIQNQQL
       :::.:::::::::: :::::::::::.:::::::::::::::::::::::::::::::::
_      KAPEYTVLQHNDQPHAVKDKITQEEGYAFFEATKLKSADATLLSSDAPVMVMAKIQNQQL
              850        860        870        880        890        900

              910        920        930        940        950        960
457676 TLSIVNPDLNLYQGREKDQFDDKGNQIEVSVYSRHWLTAESQSTNSTITVKGIWKLTTPQ
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
_      TLSIVNPDLNLYQGREKDQFDDKGNQIEVSVYSRHWLTAESQSTNSTITVKGIWKLTTPQ
              910        920        930        940        950        960

              970        980        990
457676 PGVIIKHHNNNTLITTTTIQATPTVINLVK
       :::::::::::::::::::::::::::::::
_      PGVIIKHHNNNTLITTTTIQATPTVINLVK
              970        980        990
```

SEQ ID NO: 28 Present Invention Chondroitinase ABC I nucleic acid

gccaccagcaatcctgcatttgatcctaaaaatctgatgcagtcagaaatttaccattttgcacaaaataacccattagcagacttctcatc
agataaaaactcaatactaacgttatctgataaacgtagcattatgggaaaccaatctcttttatggaaatggaaaggtggtagtagcttta
ctttacataaaaaactgattgtccccaccgataaagaagcatctaaagcatggggacgctcatccacccccgttttctcattttggctttac
aatgaaaaaccgattgatggttatcttactatcgatttcggagaaaaactcatttcaaccagtgaggctcaggcaggctttaaagtaaaatt
agatttcactggctggcgtactgtgggagtctctttaaataacgatcttgaaaatcgagagatgaccttaaatgcaaccaatacctcctctg
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tcacaacgtaaagccacaactacctgtaacacctgaaaatttagcggccattgatcttattcgccaacgtctaattaatgaatttgtcggag
gtgaaaaagagacaaacctcgcattagaagagaatatcagcaaattaaaaagtgatttcgatgctcttaatactcacactttagcaaatg
gtggaacgcaaggcagacatctgatcactgataaacaaatcattatttatcaaccagagaatcttaactctcaagataaacaactatttgat
aattatgttatttaggtaattacacgacattaatgtttaatattagccgtgcttatgtgctggaaaaagatcccacacaaaaggcgcaacta
aagcagatgtacttattaatgacaaagcatttattagatcaaggctttgttaaagggagtgctttagtgacnacccatcactggggataca
gttctcgttggtggtatatttccacgttattaatgtctgatgcactaaaagaagcgaacctacaaactcaagtttatgattcattactgtggtat
tcacgtgagtttaaaagtagttttgatatgaaagtaagtgctgatagctctgatctagattatttcaataccttatctcgccaacatttagcctt
attactactagagcctgatgatcaaaagcgtatcaacttagttaatactttcagccattatatcactggcgcattaacgcaagtgccaccgg
gtggtaaagatggtttacgccctgatggtacagcatggcgacatgaaggcaactatccgggctactctttcccagcctttaaaaatgcct
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acagtaatccagaagttggattaccgcttgcaggaagacaccctcttaactcaccttcgttaaaatcagtcgctcaaggctattactggct
tgccatgtctgcaaaatcatcgcctgataaaacacttgcatctatttatcttgcgattagtgataaaacacaaaatgaatcaactgctatttttt
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acactgaaagcttataacaccaatgtttggtcatctgaaatttataacaaagataaccgttatggccgttaccaaagtcatggtgtcgctca
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aatatggcatgatggcattcgatcttatttatcccgccaatcttgagcgttttgatcctaatttcactgcgaaaaagagtgtattagccgctg
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attaaataccctttggattaatggacaaaagatagaaaacatgccttatcaaacaacacttcaacaaggtgattggttaattgatagcaatg
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gatgactcatcgacaaaaagacactcttattgtcagtgcagttacacctgatttaaatatgactcgccaaaaagcagcaactcctgtcacc

atcaatgtcacgattaatggcaaatggcaatctgctgataaaaatagtgaagtgaaatatcaggtttctggtgataacactgaactgacgt
ttacgagttactttggtattccacaagaaatcaaactctcgccactcccttga

SEQ ID NO: 29 Present Invention Chondroitinase ABC I protein

365019 ATSNPAFDPKNLMQSEIYHFAQNNPLADFSSDKNSILTLSDKRSIMGNQSLLWKWKGGSS
::
365019 FTLHKKLIVPTDKEASKAWGRSSTPVFSFWLYNEKPIDGYLTIDFGEKLISTSEAQAGFK
365019 VKLDFTGWRTVGVSLNNDLENREMTLNATNTSSDGTQDSIGRSLGAKVDSIRFKAPSNVS
365019 QGEIYIDRIMFSVDDARYQWSDYQVKTRLSEPEIQFHNVKPQLPVTPENLAAIDLIRQRL
365019 INEFVGGEKETNLALEENISKLKSDFDALNTHTLANGGTQGRHLITDKQIIIYQPENLNS
365019 QDKQLFDNYVILGNYTTLMFNISRAYVLEKDPTQKAQLKQMYLLMTKHLLDQGFVKGSAL
365019 VTTHHWGYSSRWWYISTLLMSDALKEANLQTQVYDSLLWYSREFKSSFDMKVSADSSDLD
365019 YFNTLSRQHLALLLLEPDDQKRINLVNTFSHYITGALTQVPPGGKDGLRPDGTAWRHEGN
365019 YPGYSFPAFKNASQLIYLLRDTPFSVGESGWNSLKKAMVSAWIYSNPEVGLPLAGRHPLN
365019 SPSLKSVAQGYYWLAMSAKSSPDKTLASIYLAISDKTQNESTAIFGETITPASLPQGFYA
365019 FNGGAFGIHRWQDKMVTLKAYNTNVWSSEIYNKDNRYGRYQSHGVAQIVSNGSQLSQGYQ
365019 QEGWDWNRMPGATTIHLPLKDLDSPKPHTLMQRGERGFSGTSSLEGQYGMMAFDLIYPAN
365019 LERFDPNFTAKKSVLAADNHLIFIGSNINSSDKNKNVETTLFQHAITPTLNTLWINGQKI
365019 ENMPYQTTLQQGDWLIDSNGNGYLITQAEKVNVSRQHQVSAENKNRQPTEGNFSSAWIDH
365019 STRPKDASYEYMVFLDATPEKMGEMAQKFRENNGLYQVLRKDKDVHIILDKLSNVTGYAF
365019 YQPASIEDKWIKKVNKPAIVMTHRQKDTLIVSAVTPDLNMTRQKAATPVTINVTINGKWQ
365019 SADKNSEVKYQVSGDNTELTFTSYFGIPQEIKLSPLP

hyaluronidase 1 protein, **SEQ ID NO: 30**, Locus HSU96078
hyaluronidase 2 protein, **SEQ ID NO: 31**,
hyaluronidase 3 protein, **SEQ ID NO: 32**, Locus BC012892
hyaluronidase 4 protein, **SEQ ID NO: 33**, AF009010
PH-20 protein, **SEQ ID NO: 34**,
Tat peptide **SEQ ID NO: 35**
ABCI a site-specific mutant designated H501a and Y508a **SEQ ID NO: 36**

SEQ ID NO: 37 Chondroitinase ABCI protein Locus P59807

ORIGIN

1 mpifrftala mtlgllsapy namaatsnpa fdpknlmqse iyhfaqnnpl adfssdknsi
61 ltlsdkrsim gnqsllwkwk ggssftlhkk livptdkeas kawgrsstpv fsfwlynekp
121 idgyltidfg eklistseaq agfkvkldft gwravgvsln ndlenremtl natntssdgt
181 qdsigrslga kvdsirfkap snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf

241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnihtlan
301 ggtqgrhlit dkqiiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka
361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tllmsdalke anlqtqvyds
421 llwysrefks sfdmkvsads sdldyfntls rqhlalllle pddqkrinlv ntfshyitga
481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk
541 amvsawiysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk
601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr
661 ygryqshgva qivsngsqls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
781 vettlfqhai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq
841 hqvsaenknr qptegnfssa widhstrpkd asyeymvfld atpekmgema qkfrenngly
901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk paivmthrqk dtlivsavtp
961 dlnmtrqkaa tpvtinvtin gkwqsadkns evkyqvsgdn teltftsyfg ipqeiklspl
1021 pXX

SEQ ID NO: 38 NΔ20 ABCI (A_{45}-N_{1023}), protein

aqnnpl adfssdknsi
61 ltlsdkrsim gnqsllwkwk ggssftlhkk livptdkeas kawgrsstpv fsfwlynekp
121 idgyltidfg eklistseaq agfkvkldft gwravgvsln ndlenremtl natntssdgt
181 qdsigrslga kvdsirfkap snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnihtlan
301 ggtqgrhlit dkqiiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka
361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tllmsdalke anlqtqvyds
421 llwysrefks sfdmkvsads sdldyfntls rqhlalllle pddqkrinlv ntfshyitga
481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk
541 amvsawiysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk
601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr
661 ygryqshgva qivsngsqls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
781 vettlfqhai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq
841 hqvsaenknr qptegnfssa widhstrpkd asyeymvfld atpekmgema qkfrenngly
901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk paivmthrqk dtlivsavtp
961 dlnmtrqkaa tpvtinvtin gkwqsadkns evkyqvsgdn teltftsyfg ipqeiklspl
1021 pXX

SEQ ID NO: 39 NΔ60 ABCI (F_{85}-N_{1023}) protein

ftlhkk livptdkeas kawgrsstpv fsfwlynekp
121 idgyltidfg eklistseaq agfkvkldft gwravgvsln ndlenremtl natntssdgt
181 qdsigrslga kvdsirfkap snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnihtlan
301 ggtqgrhlit dkqiiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka
361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tllmsdalke anlqtqvyds
421 llwysrefks sfdmkvsads sdldyfntls rqhlalllle pddqkrinlv ntfshyitga
481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk
541 amvsawiysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk
601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr

661 ygryqshgva qivsngsqls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
781 vettlfqhai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq
841 hqvsaenknr qptegnfssa widhstrpkd asyeymvfld atpekmgema qkfrenngly
901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk paivmthrqk dtlivsavtp
961 dlnmtrqkaa tpvtinvtin gkwqsadkns evkyqvsgdn teltftsyfg ipqeiklspl
1021 pXX

SEQ ID NO. 40: NΔ60 CΔ80 ABCI (F_{85}-A_{942}) protein

ftlhkk livptdkeas kawgrsstpv fsfwlynekp
121 idgyltidfg eklistseaq agfkvkldft gwravgvsln ndlenremtl natntssdgt
181 qdsigrslga kvdsirfkap snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnihtlan
301 ggtqgrhlit dkqiiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka
361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tllmsdalke anlqtqvyds
421 llwysrefks sfdmkvsads sdldyfntls rqhlalllle pddqkrinlv ntfshyitga
481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk
541 amvsawiysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk
601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr
661 ygryqshgva qivsngsqls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
781 vettlfqhai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq
841 hqvsaenknr qptegnfssa widhstrpkd asyeymvfld atpekmgema qkfrenngly
901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk pa

SEQ ID NO. 41: I. Nucleotide sequence for TAT-ABCI-nΔ20:

1 ggtc gtaaaaagcg tcgtcaacgt cgtcgtcctc ctcaatgcgc acaaaataac
61 ccattagcag acttctcatc agataaaaac tcaatactaa cgttatctga taaacgtagc
121 attatgggaa accaatctct tttatggaaa tggaaaggtg gtagtagctt tactttacat
181 aaaaaactga ttgtccccac cgataaagaa gcatctaaag catggggacg ctcatccacc
241 cccgttttct cattttggct ttacaatgaa aaaccgattg atggttatct tactatcgat
301 ttcggagaaa aactcatttc aaccagtgag gctcaggcag gctttaaagt aaaattagat
361 ttcactggct ggcgtactgt gggagtctct ttaaataacg atcttgaaaa tcgagagatg
421 accttaaatg caaccaatac ctcctctgat ggtactcaag acagcattgg gcgttcttta
481 ggtgctaaag tcgatagtat tcgttttaaa gcgccttcta atgtgagtca gggtgaaatc
541 tatatcgacc gtattatgtt ttctgtcgat gatgctcgct accaatggtc tgattatcaa
601 gtaaaaactc gcttatcaga acctgaaatt caatttcaca acgtaaagcc acaactacct
661 gtaacacctg aaaatttagc ggccattgat cttattcgcc aacgtctaat taatgaattt
721 gtcggaggtg aaaaagagac aaacctcgca ttagaagaga atatcagcaa attaaaaagt
781 gatttcgatg ctcttaatac tcacacttta gcaaatggtg gaacgcaagg cagacatctg
841 atcactgata aacaaatcat tatttatcaa ccagagaatc ttaactctca agataaacaa
901 ctatttgata attatgttat tttaggtaat tacacgacat taatgtttaa tattagccgt
961 gcttatgtgc tggaaaaaga tcccacacaa aaggcgcaac taaagcagat gtacttatta
1021 atgacaaagc atttattaga tcaaggcttt gttaaaggga gtgctttagt gacnacccat
1081 cactggggat acagttctcg ttggtggtat atttccacgt tattaatgtc tgatgcacta
1141 aaagaagcga acctacaaac tcaagtttat gattcattac tgtggtattc acgtgagttt
1201 aaaagtagtt ttgatatgaa agtaagtgct gatagctctg atctagatta tttcaatacc

1261 ttatctcgcc aacatttagc cttattacta ctagagcctg atgatcaaaa gcgtatcaac
1321 ttagttaata ctttcagcca ttatatcact ggcgcattaa cgcaagtgcc accgggtggt
1381 aaagatggtt tacgccctga tggtacagca tggcgacatg aaggcaacta tccgggctac
1441 tctttcccag cctttaaaaa tgcctctcag cttatttatt tattacgcga tacaccattt
1501 tcagtgggtg aaagtggttg gaatagcctg aaaaaagcga tggtttcagc gtggatctac
1561 agtaatccag aagttggatt accgcttgca ggaagacacc ctcttaactc accttcgtta
1621 aaatcagtcg ctcaaggcta ttactggctt gccatgtctg caaaatcatc gcctgataaa
1681 acacttgcat ctatttatct tgcgattagt gataaaacac aaaatgaatc aactgctatt
1741 tttggagaaa ctattacacc agcgtcttta cctcaaggtt tctatgcctt taatggcggt
1801 gcttttggta ttcatcgttg gcaagataaa atggtgacac tgaaagctta taacaccaat
1861 gtttggtcat ctgaaattta taacaaagat aaccgttatg gccgttacca aagtcatggt
1921 gtcgctcaaa tagtgagtaa tggctcgcag ctttcacagg gctatcagca agaaggttgg
1981 gattggaata gaatgccagg ggcaaccact atccaccttc ctcttaaaga cttagacagt
2041 cctaaacctc ataccttaat gcaacgtgga gagcgtggat ttagcggaac atcatcccct
2101 gaaggtcaat atggcatgat ggcattcgat cttatttatc ccgccaatct tgagcgtttt
2161 gatcctaatt tcactgcgaa aaagagtgta ttagccgctg ataatcactt aatttttatt
2221 ggtagcaata taaatagtag tgataaaaat aaaaatgttg aaacgacctt attccaacat
2281 gccattactc caacattaaa taccctttgg attaatggac aaaagataga aaacatgcct
2341 tatcaaacaa cacttcaaca aggtgattgg ttaattgata gcaatggcaa tggttactta
2401 attactcaag cagaaaaagt aaatgtaagt cgccaacatc aggtttcagc ggaaaataaa
2461 aatcgccaac cgacagaagg aaactttagc tcggcatgga tcgatcacag cactcgcccc
2521 aaagatgcca gttatgagta tatggtcttt ttagatgcga cacctgaaaa aatgggagag
2581 atggcacaaa aattccgtga aaataatggg ttatatcagg ttcttcgtaa ggataaagac
2641 gttcatatta ttctcgataa actcagcaat gtaacgggat atgcctttta tcagccagca
2701 tcaattgaag acaaatggat caaaaaggtt aataaacctg caattgtgat gactcatcga
2761 caaaaagaca ctcttattgt cagtgcagtt acacctgatt taaatatgac tcgccaaaaa
2821 gcagcaactc ctgtcaccat caatgtcacg attaatggca aatggcaatc tgctgataaa
2881 aatagtgaag tgaaatatca ggtttctggt gataacactg aactgacgtt tacgagttac
2941 tttggtattc cacaagaaat caaactctcg ccactccctt ga

SEQ ID NO. 42: ***II. Amino acid sequence for TAT-ABCI-nΔ20:***

grkkrrqrrrppqcaqnnpladfssdknsiltlsdkrsimgnqsllwkwkggssftlhkklivptdkeaskawgrsstpvfsfw
lynekpidgyltidfgeklistseaqagfkvkldftgwrtvgvslnndlenremtlnatntssdgtqdsigrslgakvdsirfkapsnv
sqgeiyidrimfsvddaryqwsdyqvktrlsepeiqfhnvkpqlpvtpenlaaidlirqrlinefvggeketnlaleenisklksdfd
alnthtlanggtqgrhlitdkqiiiyqpenlnsqdkqlfdnyvilgnyttlmfnisrayvlekdptqkaqlkqmyllmtkhlldqgfv
kgsalvtthhwgyssrwwyistllmsdalkeanlqtqvydsllwysrefkssfdmkvsadssdldyfntlsrqhlalllllepddqkr
inlvntfshyitgaltqvppggkdglrpdgtawrhegnypgysfpafknasqliyllrdtpfsvgesgwnslkkamvsawiysnp
evglplagrhplnspslksvaqgyywlamsaksspdktlasiylaisdktqnestaifgetitpaslpqgfyafnggafgihrwqdk
mvtlkayntnvwsseiynkdnrygryqshgvaqivsngsqlsqgyqqegwdwnrmpgattihlplkdldspkphtlmqrger
gfsgtsslegqygmmafdliypanlerfdpnftakksvlaadnhlifigsninssdknknvettlfqhaitptlntlwingqkienm
pyqttlqqgdwlidsngngylitqaekvnvsrqhqvsaenknrqptegnfssawidhstrpkdasyeymvfldatpekmgema
qkfrennglyqvlrkdkdvhiildklsnvtgyafyqpasiedkwikkvnkpaivmthrqkdtlivsavtpdlnmtrqkaatpvtin
vtingkwqsadknsevkyqvsgdnteltftsyfgipqeiklsplp

SEQ ID NO. 43: II. Nucleotide sequence for TAT-ABCI-nΔ60:

ggtcgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgctttactttacataaaaaactgattgtccccaccgataaagaagcatcta
aagcatggggacgctcatccacccccgttttctcattttggctttacaatgaaaaaccgattgatggttatcttactatcgatttcggagaaa
aactcatttcaaccagtgaggctcaggcaggctttaaagtaaaattagatttcactggctggcgtactgtgggagtctctttaaataacgat
cttgaaaatcgagagatgaccttaaatgcaaccaatacctcctctgatggtactcaagacagcattgggcgttctttaggtgctaaagtcg
atagtattcgttttaaagcgccttctaatgtgagtcagggtgaaatctatatcgaccgtattatgttttctgtcgatgatgctcgctaccaatg
gtctgattatcaagtaaaaactcgcttatcagaacctgaaattcaatttcacaacgtaaagccacaactacctgtaacacctgaaaatttag
cggccattgatcttattcgccaacgtctaattaatgaatttgtcggaggtgaaaaagagacaaacctcgcattagaagagaatatcagca
aattaaaaagtgatttcgatgctcttaatactcacactttagcaaatggtggaacgcaaggcagacatctgatcactgataaacaaatcatt
atttatcaaccagagaatcttaactctcaagataaacaactatttgataattatgttatttttaggtaattacacgacattaatgtttaatattagc
cgtgcttatgtgctggaaaaagatcccacacaaaaggcgcaactaaagcagatgtacttattaatgacaaagcatttattagatcaaggc
tttgttaaagggagtgctttagtgacnacccatcactggggatacagttctcgttggtggtatatttccacgttattaatgtctgatgcactaa
aagaagcgaacctacaaactcaagtttatgattcattactgtggtattcacgtgagtttaaaagtagttttgatatgaaagtaagtgctgata
gctctgatctagattatttcaataccttatctcgccaacatttagccttattactactagagcctgatgatcaaaagcgtatcaacttagttaat
actttcagccattatatcactggcgcattaacgcaagtgccaccgggtggtaaagatggtttacgccctgatggtacagcatggcgacat
gaaggcaactatccgggctactctttcccagcctttaaaaatgcctctcagcttatttatttattacgcgatacaccattttcagtgggtgaa
agtggttggaatagcctgaaaaaagcgatggtttcagcgtggatctacagtaatccagaagttggattaccgcttgcaggaagacaccc
tcttaactcaccttcgttaaaatcagtcgctcaaggctattactggcttgccatgtctgcaaaatcatcgcctgataaaacacttgcatctatt
tatcttgcgattagtgataaaacacaaaatgaatcaactgctatttttggagaaactattacaccagcgtctttacctcaaggtttctatgcct
ttaatggcggtgcttttggtattcatcgttggcaagataaaatggtgacactgaaagcttataacaccaatgtttggtcatctgaaatttataa
caaagataaccgttatggccgttaccaaagtcatggtgtcgctcaaatagtgagtaatggctcgcagctttcacagggctatcagcaag
aaggttgggattggaatagaatgccaggggcaaccactatccaccttcctcttaaagacttagacagtcctaaacctcataccttaatgc
aacgtggagagcgtggatttagcggaacatcatcccttgaaggtcaatatggcatgatggcattcgatcttatttatcccgccaatcttga
gcgttttgatcctaatttcactgcgaaaaagagtgtattagccgctgataatcacttaatttttattggtagcaatataaatagtagtgataaa
aataaaaatgttgaaacgaccttattccaacatgccattactccaacattaaataccctttggattaatggacaaaagatagaaaacatgc
cttatcaaacaacacttcaacaaggtgattggttaattgatagcaatggcaatggttacttaattactcaagcagaaaaagtaaatgtaagt
cgccaacatcaggtttcagcggaaaataaaaatcgccaaccgacagaaggaaactttagctcggcatggatcgatcacagcactcgc
cccaaagatgccagttatgagtatatggtctttttagatgcgacacctgaaaaaatgggagagatggcacaaaaattccgtgaaaataat
gggttatatcaggttcttcgtaaggataaagacgttcatattattctcgataaactcagcaatgtaacgggatatgccttttatcagccagca
tcaattgaagacaaatggatcaaaaaggttaataaacctgcaattgtgatgactcatcgacaaaaagacactcttattgtcagtgcagtta
cacctgatttaaatatgactcgccaaaaagcagcaactcctgtcaccatcaatgtcacgattaatggcaaatggcaatctgctgataaaa
atagtgaagtgaaatatcaggtttctggtgataacactgaactgacgtttacgagttactttggtattccacaagaaatcaaactctcgcca
ctcccttga

SEQ ID NO. 44: IV. Amino acid sequence for TAT-ABCI-nΔ60

grkkrrqrrrppqcftlhkklivptdkeaskawgrsstpvfsfwlynekpidgyltidfgeklistseaqagfkvkldftgwrtvgvsl
nndlenremtlnatntssdgtqdsigrslgakvdsirfkapsnvsqgeiyidrimfsvddaryqwsdyqvktrlsepeiqfhnvkp
qlpvtpenlaaidlirqrlinefvggeketnlaleenisklksdfdalnthtlanggtqgrhlitdkqiiiyqpenlnsqdkqlfdnyvil
gnyttlmfnisrayvlekdptqkaqlkqmyllmtkhlldqgfvkgsalvtthhwgyssrwwyistllmsdalkeanlqtqvydsll
wysrefkssfdmkvsadssdldyfntlsrqhlallllepddqkrinlvntfshyitgaltqvppggkdglrpdgtawrhegnypgys
fpafknasqliyllrdtpfsvgesgwnslkkamvsawiysnpevglplagrhplnspslksvaqgyywlamsaksspdktlasiy
laisdktqnestaifgetitpaslpqgfyafnggafgihrwqdkmvtlkayntnvwsseiynkdnrygryqshgvaqivsngsqls
qgyqqegwdwnrmpgattihlplkdldspkphtlmqrgergfsgtsslegqygmmafdliypanlerfdpnftakksvlaadn
hlifigsninssdknknvettlfqhaitptlntlwingqkienmpyqttlqqgdwlidsngngylitqaekvnvsrqhqvsaenknr
qptegnfssawidhstrpkdasyeymvfldatpekmgemaqkfrennglyqvlrkdkdvhiildklsnvtgyafyqpasiedk
wikkvnkpaivmthrqkdtlivsavtpdlnmtrqkaatpvtinvtingkwqsadknsevkyqvsgdnteltftsyfgipqeiklsp
lp

SEQ ID NO. 45: V. Nucleotide sequence for ABCI-TAT-C:

gccaccagcaatcctgcatttgatcctaaaaatctgatgcagtcagaaatttaccattttgcacaaaataacccattagcagacttctcatc
agataaaaactcaatactaacgttatctgataaacgtagcattatgggaaaccaatctcttttatggaaatggaaaggtggtagtagcttta
ctttacataaaaaactgattgtccccaccgataaagaagcatctaaagcatggggacgctcatccacccccgttttctcattttggctttac
aatgaaaaaccgattgatggttatcttactatcgatttcggagaaaaactcatttcaaccagtgaggctcaggcaggctttaaagtaaaatt
agatttcactggctggcgtactgtgggagtctctttaaataacgatcttgaaaatcgagagatgaccttaaatgcaaccaatacctcctctg
atggtactcaagacagcattgggcgttctttaggtgctaaagtcgatagtattcgttttaaagcgccttctaatgtgagtcagggtgaaatc
tatatcgaccgtattatgttttctgtcgatgatgctcgctaccaatggtctgattatcaagtaaaaactcgcttatcagaacctgaaattcaatt
tcacaacgtaaagccacaactacctgtaacacctgaaaatttagcggccattgatcttattcgccaacgtctaattaatgaatttgtcggag
gtgaaaaagagacaaacctcgcattagaagagaatatcagcaaattaaaaagtgatttcgatgctcttaatactcacactttagcaaatg
gtggaacgcaaggcagacatctgatcactgataaacaaatcattatttatcaaccagagaatcttaactctcaagataaacaactatttgat
aattatgttatttttaggtaattacacgacattaatgtttaatattagccgtgcttatgtgctggaaaaagatcccacacaaaaggcgcaacta
aagcagatgtacttattaatgacaaagcatttattagatcaaggctttgttaaagggagtgctttagtgacnacccatcactggggataca
gttctcgttggtggtatatttccacgttattaatgtctgatgcactaaaagaagcgaacctacaaactcaagtttatgattcattactgtggtat
tcacgtgagtttaaaagtagttttgatatgaaagtaagtgctgatagctctgatctagattatttcaataccttatctcgccaacatttagcctt
attactactagagcctgatgatcaaaagcgtatcaacttagttaatactttcagccattatatcactggcgcattaacgcaagtgccaccgg
gtggtaaagatggtttacgccctgatggtacagcatggcgacatgaaggcaactatccgggctactctttcccagcctttaaaaatgcct
ctcagcttatttatttattacgcgatacaccattttcagtgggtgaaagtggttggaatagcctgaaaaaagcgatggtttcagcgtggatct
acagtaatccagaagttggattaccgcttgcaggaagacaccctcttaactcaccttcgttaaaatcagtcgctcaaggctattactggct
tgccatgtctgcaaaatcatcgcctgataaaacacttgcatctatttatcttgcgattagtgataaaacacaaaatgaatcaactgctatttttt
ggagaaactattacaccagcgtctttacctcaaggtttctatgcctttaatggcggtgcttttggtattcatcgttggcaagataaaatggtg
acactgaaagcttataacaccaatgtttggtcatctgaaatttataacaaagataaccgttatggccgttaccaaagtcatggtgtcgctca
aatagtgagtaatggctcgcagctttcacagggctatcagcaagaaggttgggattggaatagaatgccaggggcaaccactatccac
cttcctcttaaagacttagacagtcctaaacctcataccttaatgcaacgtggagagcgtggatttagcggaacatcatcccttgaaggtc
aatatggcatgatggcattcgatcttatttatcccgccaatcttgagcgttttgatcctaatttcactgcgaaaaagagtgtattagccgctg
ataatcacttaatttttattggtagcaatataaatagtagtgataaaaataaaaatgttgaaacgaccttattccaacatgccattactccaac
attaaataccctttggattaatggacaaaagatagaaaacatgccttatcaaacaacacttcaacaaggtgattggttaattgatagcaatg
gcaatggttacttaattactcaagcagaaaaagtaaatgtaagtcgccaacatcaggtttcagcggaaaataaaaatcgccaaccgaca
gaaggaaactttagctcggcatggatcgatcacagcactcgccccaaagatgccagttatgagtatatggtctttttagatgcgacacct
gaaaaaatgggagagatggcacaaaaattccgtgaaaataatgggttatatcaggttcttcgtaaggataaagacgttcatattattctcg
ataaactcagcaatgtaacgggatatgccttttatcagccagcatcaattgaagacaaatggatcaaaaaggttaataaacctgcaattgt
gatgactcatcgacaaaaagacactcttattgtcagtgcagttacacctgatttaaatatgactcgccaaaaagcagcaactcctgtcacc
atcaatgtcacgattaatggcaaatggcaatctgctgataaaaatagtgaagtgaaatatcaggtttctggtgataacactgaactgacgt
ttacgagttactttggtattccacaagaaatcaaactctcgccactccct
ggtcgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgctag

SEQ ID NO. 46: V. Amino acid sequence for ABCI-TAT-C:

atsnpafdpknlmqseiyhfaqnnpladfssdknsiltlsdkrsimgnqsllwkwkggssftlhkklivptdkeaskawgrsstpv
fsfwlynekpidgyltidfgeklistseaqagfkvkldftgwrtvgvslnndlenremtlnatntssdgtqdsigrslgakvdsirfka
psnvsqgeiyidrimfsvddaryqwsdyqvktrlsepeiqfhnvkpqlpvtpenlaaidlirqrlinefvggeketnlaleenisklk
sdfdalnthtlanggtqgrhlitdkqiiiyqpenlnsqdkqlfdnyvilgnyttlmfnisrayvlekdptqkaqlkqmyllmtkhlld
qgfvkgsalvtthhwgyssrwwyistllmsdalkeanlqtqvydsllwysrefkssfdmkvsadssdldyfntlsrqhlallllepd
dqkrinlvntfshyitgaltqvppggkdglrpdgtawrhegnypgysfpafknasqliyllrdtpfsvgesgwnslkkamvsawi
ysnpevglplagrhplnspslksvaqgyywlamsaksspdktlasiylaisdktqnestaifgetitpaslpqgfyafnggafgihr
wqdkmvtlkayntnvwsseiynkdnrygryqshgvaqivsngsqlsqgyqqegwdwnrmpgattihlplkdldspkphtlm
qrgergfsgtsslegqygmmafdliypanlerfdpnftakksvlaadnhlifigsninssdknknvettlfqhaitptlntlwingqki

enmpyqttlqqgdwlidsngngylitqaekvnvsrqhqvsaenknrqptegnfssawidhstrpkdasyeymvfldatpekm
gemaqkfrennglyqvlrkdkdvhiildklsnvtgyafyqpasiedkwikkvnkpaivmthrqkdtlivsavtpdlnmtrqkaa
tpvtinvtingkwqsadknsevkyqvsgdnteltftsyfgipqeiklsplpgrkkrrqrrrppqc

SEQ ID NO. 47 Nucleotide sequence for chondroitinase ABCI-nΔ20

gc acaaaataac
61 ccattagcag acttctcatc agataaaaac tcaatactaa cgttatctga taaacgtagc
121 attatgggaa accaatctct tttatggaaa tggaaaggtg gtagtagctt tactttacat
181 aaaaaactga ttgtccccac cgataaagaa gcatctaaag catggggacg ctcatccacc
241 cccgttttct cattttggct ttacaatgaa aaaccgattg atggttatct tactatcgat
301 ttcggagaaa aactcatttc aaccagtgag gctcaggcag gctttaaagt aaaattagat
361 ttcactggct ggcgtactgt gggagtctct ttaaataacg atcttgaaaa tcgagagatg
421 accttaaatg caaccaatac ctcctctgat ggtactcaag acagcattgg gcgttcttta
481 ggtgctaaag tcgatagtat tcgttttaaa gcgccttcta atgtgagtca gggtgaaatc
541 tatatcgacc gtattatgtt ttctgtcgat gatgctcgct accaatggtc tgattatcaa
601 gtaaaaactc gcttatcaga acctgaaatt caatttcaca acgtaaagcc acaactacct
661 gtaacacctg aaaatttagc ggccattgat cttattcgcc aacgtctaat taatgaattt
721 gtcggaggtg aaaaagagac aaacctcgca ttagaagaga atatcagcaa attaaaaagt
781 gatttcgatg ctcttaatac tcacacttta gcaaatggtg gaacgcaagg cagacatctg
841 atcactgata aacaaatcat tatttatcaa ccagagaatc ttaactctca agataaacaa
901 ctatttgata attatgttat tttaggtaat tacacgacat taatgtttaa tattagccgt
961 gcttatgtgc tggaaaaaga tcccacacaa aaggcgcaac taaagcagat gtacttatta
1021 atgacaaagc atttattaga tcaaggcttt gttaaaggga gtgctttagt gacnacccat
1081 cactggggat acagttctcg ttggtggtat atttccacgt tattaatgtc tgatgcacta
1141 aaagaagcga acctacaaac tcaagtttat gattcattac tgtggtattc acgtgagttt
1201 aaaagtagtt ttgatatgaa agtaagtgct gatagctctg atctagatta tttcaatacc
1261 ttatctcgcc aacatttagc cttattacta ctagagcctg atgatcaaaa gcgtatcaac
1321 ttagttaata ctttcagcca ttatatcact ggcgcattaa cgcaagtgcc accgggtggt
1381 aaagatggtt tacgccctga tggtacagca tggcgacatg aaggcaacta tccgggctac
1441 tctttcccag cctttaaaaa tgcctctcag cttatttatt tattacgcga tacaccattt
1501 tcagtgggtg aaagtggttg gaatagcctg aaaaaagcga tggtttcagc gtggatctac
1561 agtaatccag aagttggatt accgcttgca ggaagacacc ctcttaactc accttcgtta
1621 aaatcagtcg ctcaaggcta ttactggctt gccatgtctg caaaatcatc gcctgataaa
1681 acacttgcat ctatttatct tgcgattagt gataaaacac aaaatgaatc aactgctatt
1741 tttggagaaa ctattacacc agcgtcttta cctcaaggtt tctatgcctt taatggcggt
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2221 ggtagcaata taaatagtag tgataaaaat aaaaatgttg aaacgacctt attccaacat
2281 gccattactc caacattaaa tacccctttgg attaatggac aaaagataga aaacatgcct
2341 tatcaaacaa cacttcaaca aggtgattgg ttaattgata gcaatggcaa tggttactta
2401 attactcaag cagaaaaagt aaatgtaagt cgccaacatc aggtttcagc ggaaaataaa
2461 aatcgccaac cgacagaagg aaactttagc tcggcatgga tcgatcacag cactcgcccc
2521 aaagatgcca gttatgagta tatggtcttt ttagatgcga cacctgaaaa aatgggagag
2581 atggcacaaa aattccgtga aaataatggg ttatatcagg ttcttcgtaa ggataaagac

2641 gttcatatta ttctcgataa actcagcaat gtaacgggat atgcctttta tcagccagca
2701 tcaattgaag acaaatggat caaaaaggtt aataaacctg caattgtgat gactcatcga
2761 caaaaagaca ctcttattgt cagtgcagtt acacctgatt taaatatgac tcgccaaaaa
2821 gcagcaactc ctgtcaccat caatgtcacg attaatggca aatggcaatc tgctgataaa
2881 aatagtgaag tgaaatatca ggtttctggt gataacactg aactgacgtt tacgagttac
2941 tttggtattc cacaagaaat caaactctcg ccactccctt ga

SEQ ID NO. 48: II. Nucleotide sequence for ABCI-nΔ60:

tttactttacataaaaaactgattgtccccaccgataaagaagcatctaaagcatggggacgctcatccacccccgttttctcattttggctt
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caccatcaatgtcacgattaatggcaaatggcaatctgctgataaaaatagtgaagtgaaatatcaggtttctggtgataacactgaactg
acgtttacgagttactttggtattccacaagaaatcaaactctcgccactcccttga

SEQ ID NO. 49: Nucleotide sequence for TAT

ggtcgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgc

(SEQ ID NO. 50) Amino acid sequence for a TAT peptide

grkkrrqrrrppqc